FINAL REPORT

Volume 4 of 4
(Appendices C - J)

STUDY TITLE

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY

STUDY NUMBER

WIL-189207

DATA REQUIREMENT

OECD Guideline 407

STUDY DIRECTOR

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STUDY INITIATION DATE

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PERFORMING LABORATORY

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APPENDIX C

Analyses Of Dosing Formulations (WIL Research Laboratories, LLC)

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY OF H-28397 IN MICE WITH A 28-DAY RECOVERY

Analysis Of Dosing Formulations

Analytical Chemistry Department

WIL Research Laboratories, LLC

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TABLE OF CONTENTS

Page

INDEX OF FIGURES

INDEX OF TABLES

INDEX OF ATTACHMENTS

REFERENCES

Haas, M. C. A 28-Day Oral (Gavage) Toxicity Study Of H-28397 In Rats With A 28-Day Recovery. (Study No. WIL-189205). WIL Research Laboratories, LLC, Ashland, OH, 2008.

SUMMARY

A high performance liquid chromatography tandem mass spectrometry method in the negative electrospray ionization mode for the determination of H-28397 concentration in aqueous formulations containing deionized (DI) water and test article ranging in concentration from 0.00100 to 50.0 mg/mL was validated in a previous study (Haas, 2008). Also in that study, it was concluded that results of the assessments were excessively variable due to unstable ionization during the analyses. Consequently, on 21 December 2007, the assay was cross-validated in that study for use with modified diluent and mobile phases. In the present study, preinitiation (prepared prior to dosing and not used for dosing) formulations prepared at target concentrations of 0.01 and 3 mg H-28397/mL were evaluated for test article homogeneity and, following room temperature storage for 5 hours and refrigerated 12 days, resuspension homogeneity and stability. Formulations prepared at target concentrations of 0, 0.01, 0.3 and 3 mg H-28397/mL and used for dose administration were analyzed to confirm test article concentration. Quantitation was performed using calibration standards ranging in test article concentration from 100 to 1000 ng/mL. Assay precision and accuracy were verified by the analysis of quality control samples prepared at 0.00100, 0.0500, 0.500 and 50.0 mg/mL.

Fomulations prepared at target test article concentrations of 0.01 and 3 mg/mL were analyzed to assess test article homogeneity and, following room temperature storage for 5 hours and refrigerated 12 days, test article resuspension homogeneity and stability. The assessments met the WIL standard operating procedures (SOP) acceptance criteria for test article homogeneity, i.e., the variability for the mean concentration was ≤ 10% relative standard deviation (RSD) at a concentration within the acceptable limits (85% to 115% of target), resuspension homogeneity, i.e., the variability for the mean concentration was ≤ 10% RSD, and for stability, i.e., the post-storage concentration was not less than 90% of the pre-storage value, with the following exception. The 13 and14 December 2007 formulation prepared at the target test article concentration of 3 mg/mL and stored at room temperature for 5 hours failed to meet the WIL SOP requirement for resuspension homogeneity (11% RSD). The out-of-specification results were believed to be a result of drift in ionization efficiency in the mass spectrometer. Consequently, the study director evaluated the data and considered it acceptable. To reduce the variability, the assay was cross-validated with a modified diluent and mobile phases in another study (Haas, 2008) prior to the 12-day resuspension homogeneity assessment.

Dosing formulations prepared at target test article concentrations of 0, 0.01, 0.3 and 3 mg/mL were analyzed to confirm test article concentration acceptability. The results met the WIL SOP acceptance criteria for concentration acceptability in suspension formulations, i.e., the mean concentration was 85% to 115% of the target concentration with the following exception. The 17-18 December 2007 Group 3 formulation prepared at the target test article concentration of 0.3 mg/mL was 77.8% of target. Consequently, the study director changed the dose volume from 10 mL/kg to 12 mL/kg for that Group 3 formulaton. No test article was detected in the vehicle used for administration to the control group.

INTRODUCTION

This report provides a detailed description of a high performance liquid chromatography tandem mass spectrometry (HPLC/MS/MS) method in the negative electrospray ionization (ESI-) mode for the determination of H-28397 concentration in aqueous formulations containing deionized (DI) water and test article ranging in concentration from 0.00100 to 50.0 mg/mL. Method specificity/selectivity, ruggedness, calibration reproducibility, precision and accuracy were assessed and validated in a separate study (Haas, 2008). In the present study, Formulations prepared at target test article concentrations of 0.01 and 3 mg/mL were analyzed to assess homogeneity and, following storage at room temperature for 5 hours and refrigerated 12 days, test article resuspension homogeneity and stability. Dosing formulations prepared at target test article concentrations of 0, 0.01, 0.3 and 3 mg/mL were analyzed to confirm test article concentration acceptability.

EXPERIMENTAL (INITIAL METHOD)

A. Instruments

The HPLC/MS/MS system used was a Waters 2695 liquid chromatograph equipped with an autosampler and a Micromass Quattro Micro™ triple quadrupole mass spectrometer equipped with an ESI- interface. Data acquisition and analysis were performed using MassLynx™ software version 4.1 or equivalent. The retention time, run time and mass spectrometer settings may have varied depending on column and mass spectrometer performance.

1. High Performance Liquid Chromatography

Instrument:	Waters 2695 liquid chromatograph equipped with an autosampler, Micromass tandem quadrupole Quattro Micro™ Mass Spectrometer and MassLynx™ software or equivalent system
Column:	Phenomenex Synergi Polar-RP 4 µm 75 × 2.0 mm
Column Temperature:	40ºC
Mobile Phase:	A: 0.15% (v/v) glacial acetic acid in DI Water.
	B: 0.15% (v/v) glacial acetic acid in acetonitrile.
Composition:	35% A, 65% B, (v/v)
Flow Rate:	0.4 mL/minute
Detector:	Mass spectrometer with conditions as described in Experimental (Initial Method) A.2.(Mass Spectrometry)
Injection Volume:	10.0 µL
Retention Time:	Approximately 0.5 to 0.6 minutes for H-28397
Run Time:	1.5 minutes
Injector Wash:	90:10 ACN:DI water

2. Mass Spectrometry

Ion Mode:	ESI-
Capillary Voltage:	1.5 kV
Cone:	9.00 V
Extractor:	3.00 V
RF Lens:	0.4 V
Source Temperature:	100°C
Desolvation Temperature:	400°C
Cone Gas Flow:	Approximately 100 L nitrogen/hour
Desolvation Gas Flow:	Approximately 700 L nitrogen/hour

Acquisition Parameters

Function Type:	Multiple reaction monitoring
Precursor/Product Ion:	m/z 328.85/284.85 for H-28397
Collision Gas:	Argon
Collision Cell Pressure:	Approximately 3.28×10^{-3} mbar
Collision Energy:	5.0 V

B. Mobile Phase A Preparation

Mobile phase A was prepared by thoroughly mixing glacial acetic acid (GAA; 1.5 mL) in a final volume of 1000-mL of vacuum-degassed DI water. The preparation was scaled as needed.

C. Mobile Phase B Preparation

Mobile phase B was prepared by thoroughly mixing GAA (1.5 mL) in a final volume of 1000-mL of acetonitrile (ACN). The preparation was scaled as needed.

D. Preparation Of Calibration Stock Solution

A calibration stock solution was prepared at a concentration of 1 mg H-28397/mL as follows. Approximately 114 mg H-28397 (WIL log no. 7741, purity 88.0%) was accurately weighed in a 100-mL volumetric flask. DI water was added, and the preparation was stirred to achieve

complete dissolution. Additional DI water was added to achieve the desired concentration, and the solution was stirred to mix.

E. Preparation Of Secondary Calibration Stock Solution

A secondary calibration stock solution was prepared at a concentration of 0.00100 mg H-28397/mL as follows. An aliquot of the calibration stock solution was diluted 1000-fold with DI water, and the solution was stirred to mix. The secondary stock solution was prepared fresh as needed.

F. Preparation Of Calibration Samples

Calibration samples at test article concentrations of 100, 250, 500, 750 and 1000 ng H-28397/mL were prepared in triplicate for analysis by diluting aliquots of the secondary calibration stock solution with DI water in amber autosampler vials.

G. Preparation Of Quality Control Stock Solution

The quality control (QC) stock solution was prepared at 50 mg H-28397/mL by accurately weighing approximately 0.57 g of H-28397 (WIL log no.7741, purity 88.0%) in a tared 10-mL volumetric flask. The flask was partially filled with DI water and stirred to achieve complete dissolution. The solution was brought to volume with DI water and stirred to mix.

H. Preparation Of Secondary QC Stock Solution

A secondary QC stock solution was prepared at a concentration of 0.100 mg H-28397 /mL as follows. An aliquot of the QC stock solution was diluted 500 fold with DI water, and the solution was stirred to mix. The secondary stock solution was prepared fresh as needed.

I. Preparation Of Quality Control Samples

As detailed in the following table, the lowest (LLQC), low (LQC), middle (MQC) and high concentration (HQC) QC samples were prepared at 0.00100, 0.0500, 0.500 and 50.0 mg H-28397/mL, respectively, by thoroughly mixing aliquots of the QC stock or secondary QC stock solutions and DI water in polypropylene tubes.

	Stock Concentration (mg/mL)	Stock Volume (mL)	DI Water Volume (mL)	Total Volume (mL)	QC Concentration (mg/mL)
Blank	0	0	10.0	10.0	0
LLQC	0.100	0.100	9.90	10.0	0.00100
LQC	0.100	5.00	5.00	10.0	0.0500
MQC	50.0	0.100	9.90	10.0	0.500
HQC	50.0	1.00	0	1.00	50.0

J. QC Sample Processing

As detailed in the following table, aliquots of the LLQC, LQC, MQC and HQC samples and DI water were thoroughly mixed in amber autosampler vials (blank and LLQC samples) or polypropylene tubes (LQC, MQC and HQC samples). Aliquots of the MQC and HQC diluted samples were further diluted with DI water, and the samples were mixed by vortex action. Portions of the processed LQC, MQC and HQC samples were transferred to amber autosampler vials for analysis.

	Initial Concentration (mg/mL)	QC Sample Aliquot (mL)	DI Water Volume (mL)	Secondary Dilution	Diluted Concentration (µg/mL)
Blank	0	1.00	0	NA	0
LLQC	0.00100	0.500	0.500	NA	0.500
LQC	0.0500	0.100	9.900	NA	0.500
MQC	0.500	0.100	9.900	10-fold	0.500
HQC	50.0	0.100	9.900	1000-fold	0.500

NA = Not applicable

K. Sample Processing

As detailed in the following tables, formulation samples (1.0 mL each) were diluted with DI water in polypropylene tubes. The samples were thoroughly mixed, and portions of the diluted Group 1 and 2 samples were transferred into amber autosampler vials for analysis. Secondary dilutions with DI water were performed on the remaining samples as necessary to achieve a final test article concentration of 0.500 µg/mL. Portions of the processed samples were transferred to amber autosampler vials for analysis .

Groups	Target Concentration (µg/mL)	Sample Volume (mL)	DI Water Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/mL)
1	0	1.0	9.00	10	0
2	10.0	1.0	9.00	10	1.00
3	300	1.0	39.00	40	7.50
4	3000	1.0	39.00	40	75.0

Note: Alternate dilutions may be used.

Secondary Dilutions:

Group	Initial Diluted Concentration (µg/mL)	Aliquot Volume (mL)	DI Water Volume (mL)	Final Volume (mL)	Theoretical Final Concentration (µg/mL)
2	1.00	0.500	0.500	1.00	0.500
3	7.50	0.100	1.400	1.50	0.500
4	75.0	0.100	14.900	15.0	0.500

L. Preparation Of Backup Samples

Formulation samples (1.0 mL each) were collected and stored in polypropylene tubes. The samples were stored frozen (approximately -20°C) in the Analytical Chemistry Department.

M. Concentration Quantitation

Single injections were made of each calibration standard and processed QC and formulation sample. A calibration curve was constructed for each set of analysis. Using MassLynx™, the H-28397 peak areas (y) and the theoretical concentrations of the calibration standards (x) were fit to the ln-quadratic function using least-squares regression analysis:

$$\ln (y) = a \times [\ln (x)]^2 + b \times \ln (x) + c$$

Concentration and percent relative error (%RE) were calculated using MassLynx™. The concentration data were transferred to an Excel spreadsheet, where appropriate summary statistics, i.e., mean, standard deviation (SD), relative standard deviation (RSD), percent relative error (%RE) and percent of target, were calculated and presented in tabular form. The concentrations of the dosing formulations and QC samples were calculated by applying any necessary multiplication factors.

EXPERIMENTAL (MODIFIED METHOD)

A. Instruments

The HPLC/MS/MS system used was a Waters 2695 liquid chromatograph equipped with an autosampler and a Micromass Quattro Micro™ triple quadrupole mass spectrometer equipped with an ESI- interface. Data acquisition and analysis were performed using MassLynx™ software version 4.1 or equivalent. The retention time, run time and mass spectrometer settings may have varied depending on column and mass spectrometer performance.

1. High Performance Liquid Chromatography

Instrument:	Waters 2695 liquid chromatograph equipped with an autosampler, Micromass tandem quadrupole Quattro Micro™ Mass Spectrometer and MassLynx™ software or equivalent system
Column:	Phenomenex Synergi Polar-RP 4 µm 75 × 2.0 mm
Column Temperature:	40ºC
Mobile Phase:	A: 90:10 (v/v) 5 mM ammonium acetate in DI water, pH 2.5: ACN B: 10:90 (v/v) 5 mM ammonium acetate in DI water, pH 2.5: acetonitrile
Composition:	50% A, 50% B(v/v)
Flow Rate:	0.4 mL/minute
Detector:	Mass spectrometer with conditions as described in Experimental (Modified Method) A.2. (Mass Spectrometry)
Injection Volume:	10 µL
Retention Time:	Approximately 0.5 to 0.6 minutes for H-28397
Run Time:	1.0 minutes
Injector Wash:	90:10 (v/v) ACN:DI water

2. Mass Spectrometry

Ion Mode:	ESI-
Capillary Voltage:	1.50 kV
Cone:	9.00 V
Extractor:	3.00 V
RF Lens:	0.4 V
Source Temperature:	100°C
Desolvation Temperature:	400°C
Cone Gas Flow:	Approximately 100 L nitrogen/hour
Desolvation Gas Flow:	Approximately 700 L nitrogen/hour

Acquisition Parameters

Function Type:	Multiple reaction monitoring
Precursor/Product Ion:	m/z 328.85/284.85 for H-28397
Collision Gas:	Argon
Collision Cell Pressure:	Approximately 3.28×10^{-3} mbar
Collision Energy:	5.0 V

B. Preparation Of Diluent And Buffer (5 mM Ammonium Acetate In DI Water, pH 2.5)

Ammonium acetate (0.77g) was dissolved in a final volume of 2 liters of DI water and vacuum-filtered through a 0.45-µm nylon filter. The solution was transferred into a storage bottle, and the pH was adjusted to approximately 2.5 with GAA. The solution was also used as a buffer for preparation of mobile phase A and mobile phase B. In addition, the solution was used as the diluent in the preparation of the calibration and QC stock solutions and the processing of the QC and formulation samples. The preparation was scaled as needed.

C. Mobile Phase A Preparation (90:10 [v/v] 5 mM Ammonium Acetate In DI Water, pH 2.5: Acetonitrile)

Ammonium acetate buffer (900 mL) and 100 mL of ACN were thoroughly mixed and degassed by sonication. The solution was stored at room temperature for up to one month. This preparation was scaled as needed.

D. Mobile Phase B Preparation (10:90 5 mM Ammonium Acetate In DI Water, pH 2.5: Acetonitrile)

Ammonium acetate buffer (100 mL) and 900 mL of ACN were thoroughly mixed and degassed by sonication. The solution was stored at room temperature for up to one month. The preparation was scaled as needed.

E. Preparation Of Calibration Stock Solution

A calibration stock solution was prepared at a concentration of 1 mg H-28397/mL as follows. Approximately 114 mg H-28397 (WIL log no. 7741, purity 88.0%) was accurately weighed in a 100-mL volumetric flask. Diluent was added, and the preparation was stirred to achieve complete dissolution. Additional diluent was added to achieve the desired concentration, and the solution was stirred to mix.

F. Preparation Of Secondary Calibration Stock Solution

A secondary calibration stock solution was prepared at a concentration of 0.00100 mg H-28397/mL as follows. An aliquot of the calibration stock solution was diluted 1000-fold with diluent. The secondary calibration stock solution was prepared fresh as needed.

G. Preparation Of Calibration Samples

Calibration samples at test article concentrations of 100, 250, 500, 750 and 1000 ng H-28397/mL were prepared in triplicate for analysis by diluting aliquots of the secondary calibration stock solution with diluent in amber autosampler vials.

H. Preparation Of Quality Control Stock Solution

The quality control (QC) stock solution was prepared at 50 mg H-28397/mL by accurately weighing approximately 0.57 g of H-28397 (WIL log no.7741, purity 88.0%) in a tared 10-mL volumetric flask. The flask was partially filled with diluent and stirred to achieve complete dissolution. The solution was brought to volume with diluent and stirred to mix.

I. Preparation Of Secondary QC Stock Solution

A secondary QC stock solution was prepared at a concentration of 0.100 mg H-28397/mL as follows. An aliquot of the QC stock solution was diluted 500-fold with diluent, and the solution was stirred to mix. The secondary QC stock solution was prepared fresh as needed.

J. Preparation Of Quality Control Samples

As detailed in the following table, the LLQC, LQC, MQC and HQC samples were prepared at 0.00100, 0.0500, 0.500 and 50.0 mg H-28397/mL, respectively, by thoroughly mixing aliquots of the QC stock or secondary QC stock solutions and diluent in polypropylene tubes.

	QC Stock Concentration (mg/mL)	DI Water Volume (mL)	Diluent Volume (mL)	QC Stock Volume (mL)	Total Volume (mL)	QC Concentration (mg/mL)
Blank	0	1.00	9.00	0	10.0	0
LLQC	0.100	1.00	8.90	0.100	10.0	0.00100
LQC	0.100	1.00	4.00	5.00	10.0	0.0500
MQC	50.0	1.00	8.90	0.100	10.0	0.500
HQC	50.0	0	0	1.00	1.00	50.0

K. QC Sample Processing

As detailed in the following table, aliquots of the LLQC, LQC, MQC and HQC samples and diluent were thoroughly mixed in amber autosampler vials (blank and LLQC samples) or polypropylene tubes (LQC, MQC and HQC samples). Aliquots of the MQC and HQC diluted samples were further diluted with diluent, and the samples were mixed by vortex action. Portions of the processed LQC, MQC and HQC samples were transferred to amber autosampler vials for analysis.

	Initial Concentration (mg/mL)	QC Sample Aliquot (mL)	Diluent Volume (mL)	Secondary Dilution	Diluted Concentration (µg/mL)
Blank	0	0.500	0.500	NA	0
LLQC	0.00100	0.500	0.500	NA	0.500
LQC	0.0500	0.100	9.900	NA	0.500
MQC	0.500	0.100	9.900	10-fold	0.500
HQC	50.0	0.100	9.900	1000-fold	0.500

NA = Not applicable

L. Sample Processing

As detailed in the following tables, formulation samples (1.0 mL each) were diluted with diluent in 50-mL polypropylene tubes. The samples were thoroughly mixed, and portions of the diluted Group 1 and 2 samples were transferred into amber autosampler vials for analysis. Secondary dilutions were performed with diluent on the remaining samples as necessary to achieve a final test article concentration of 0.500 µg/mL, and portions of the processed samples were transferred to amber autosampler vials for analysis.

Groups	Target Concentration (µg/mL)	Sample Volume (mL)	Diluent Volume (mL)	Total Volume (mL)	Diluted Concentration (µg/mL)
1	0	1.0	9.00	10	0
2	10.0	1.0	19.00	20	0.500
3	300	1.0	39.00	40	7.50
4	3000	1.0	39.00	40	75.0

Secondary Dilutions:

Group	Initial Diluted Concentration (µg/mL)	Aliquot Volume (mL)	Diluent Volume (mL)	Final Volume (mL)	Theoretical Final Concentration (µg/mL)
3	7.50	0.100	1.400	1.50	0.500
4	75.0	0.100	14.900	15.0	0.500

Note: Alternate dilutions may be used.

M. Preparation Of Backup Samples

Formulation samples (1.0 mL each) were collected and stored in polypropylene tubes. The samples were stored frozen (-20°C) in the Analytical Chemistry Department.

N. Concentration Quantitation

Single injections were made of each calibration standard and processed QC and formulation sample. A calibration curve was constructed for each set of analysis. Using MassLynx™, the H-28397 peak areas (y) and the theoretical concentrations of the calibration standards (x) were fit to the ln-quadratic function using least-squares regression analysis:

$$\ln (y) = a \times [\ln (x)]^2 + b \times \ln (x) + c$$

Concentration and RE were calculated using MassLynx™. The concentration data were transferred to an Excel spreadsheet, where appropriate summary statistics, i.e., mean, SD, RSD, %RE and percent of target, were calculated and presented in tabular form. The concentrations of the dosing formulations and QC samples were calculated by applying any necessary multiplication factors.

RESULTS AND DISCUSSION

Under the described chromatographic conditions, the retention time of the test article was approximately 0.5 to 0.6 minutes. Figures 1, 2, 3 and 4 are typical chromatograms of a processed calibration standard, a processed QC sample, a processed formulation sample, and a processed blank QC sample, respectively, using the initial assay. Figures 5, 6, 7 and 8 are typical chromatograms of a calibration standard, a processed QC sample, a processed formulation sample, and a processed vehicle sample, respectively, using the modified assay. The total analysis time required for each run was 1.5 minutes for the initial assay and 1.0 minute for the modified assay.

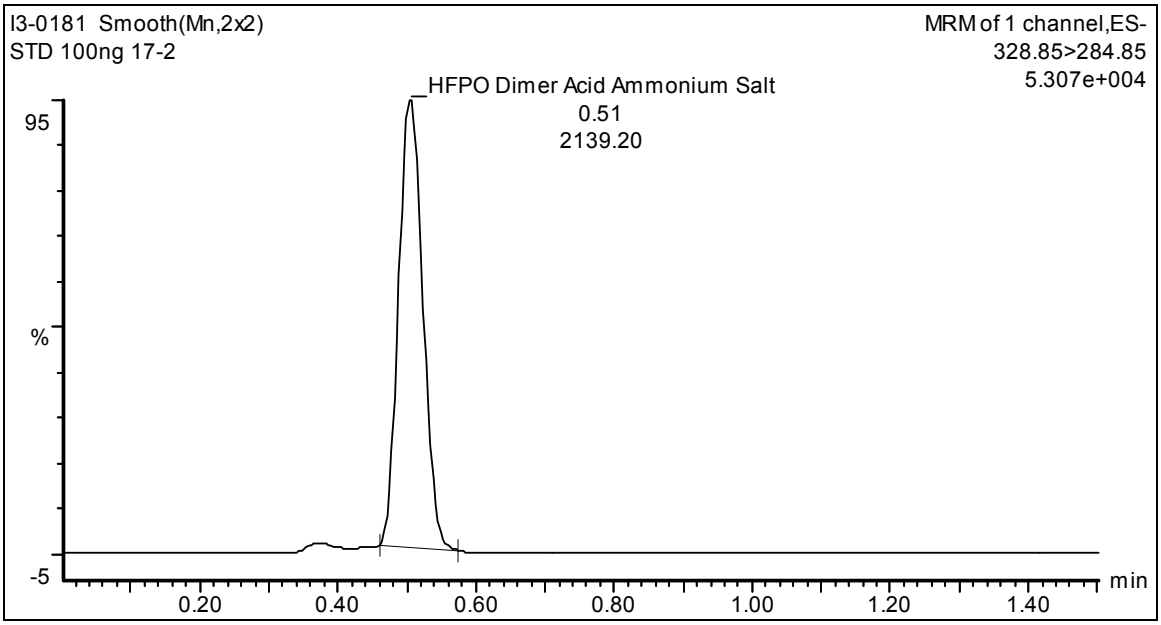

Figure 1: Representative Chromatogram Of A 100 ng H-28397/mL Calibration Standard

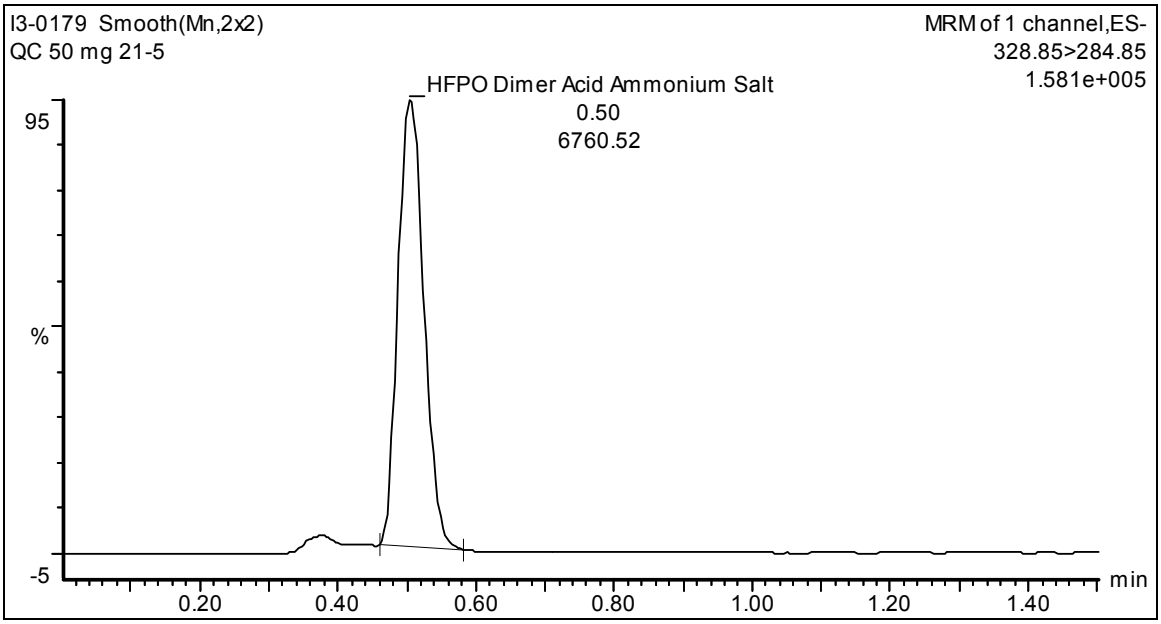

Figure 2: Representative Chromatogram Of A Processed 50 mg H-28397/mL Quality Control Sample

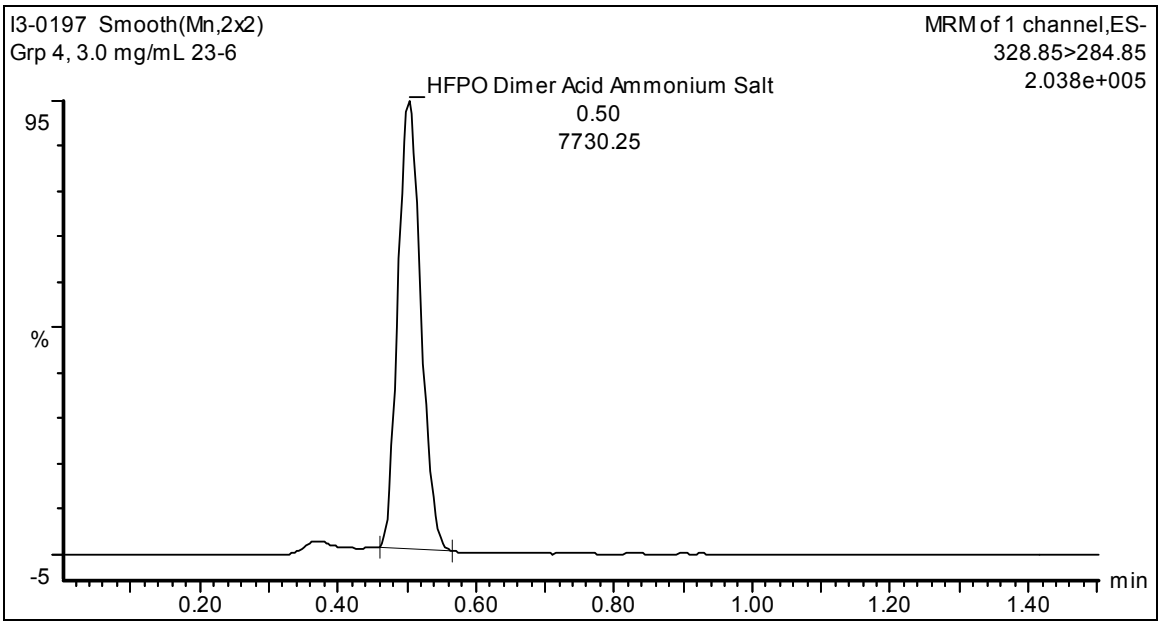

Figure 3: Representative Chromatogram Of A Processed 3 mg H-28397/mL Formulation Sample

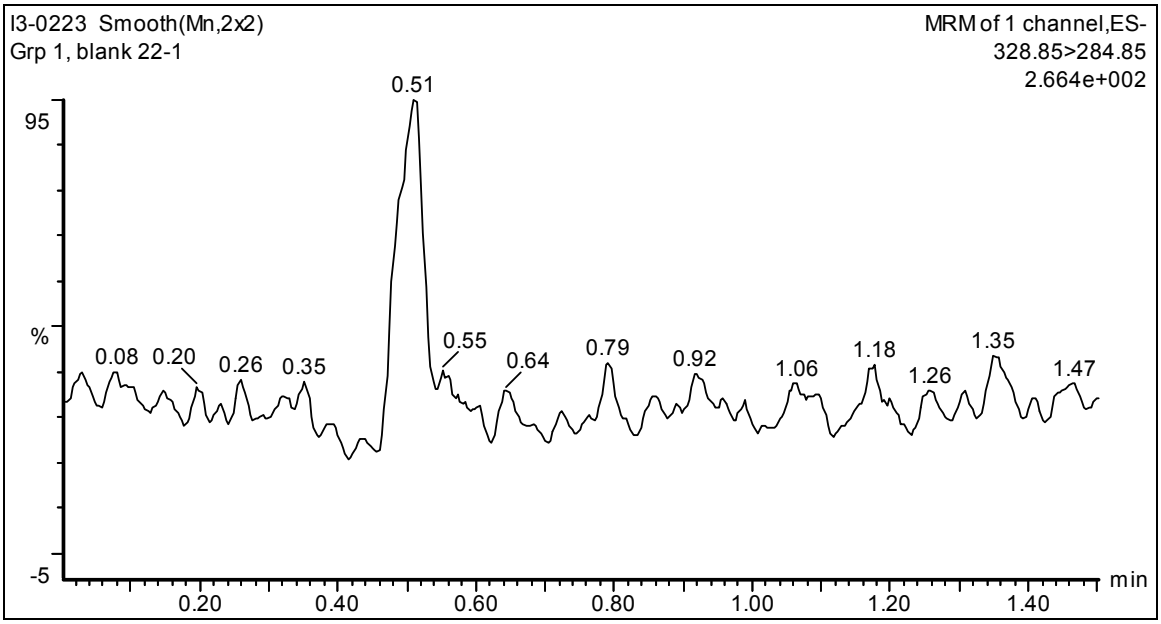

Figure 4: Representative Chromatogram Of A Processed Control Formulation Sample

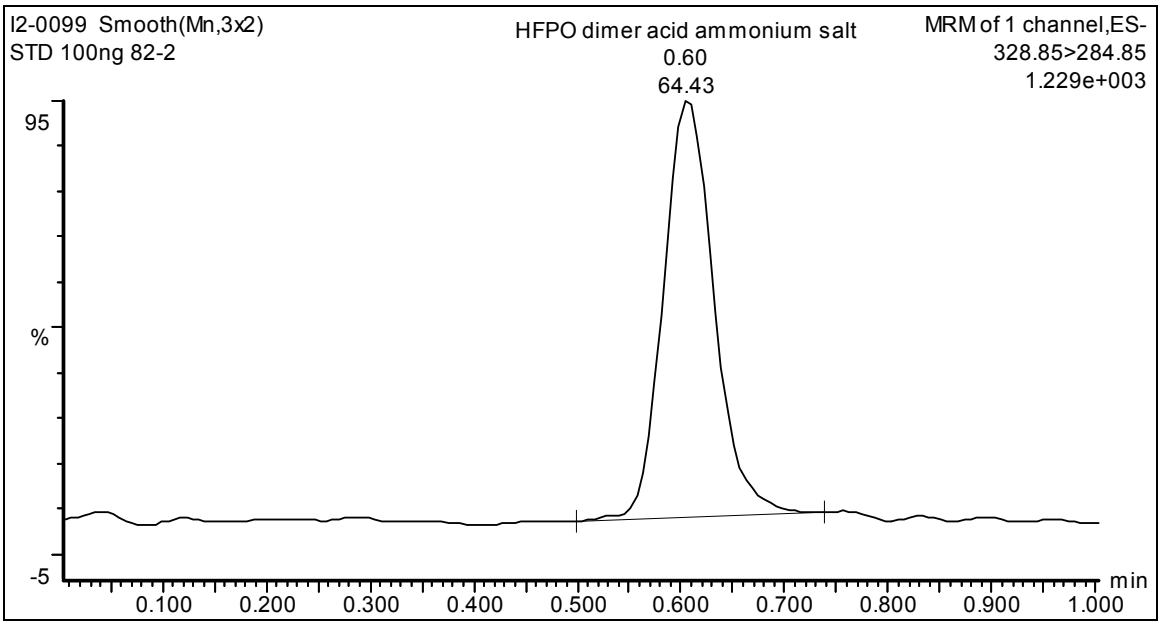

Figure 5: Representative Chromatogram Of A 100 ng H-28397/mL Calibration Standard

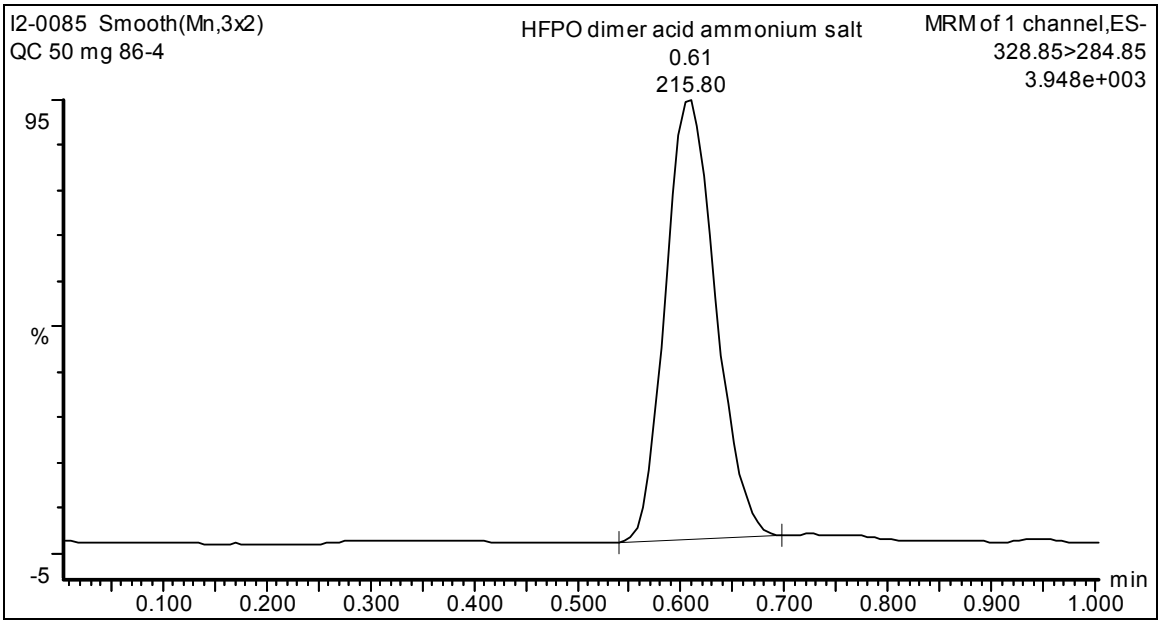

Figure 6: Representative Chromatogram Of A Processed 50 mg H-28397/mL Quality Control Sample

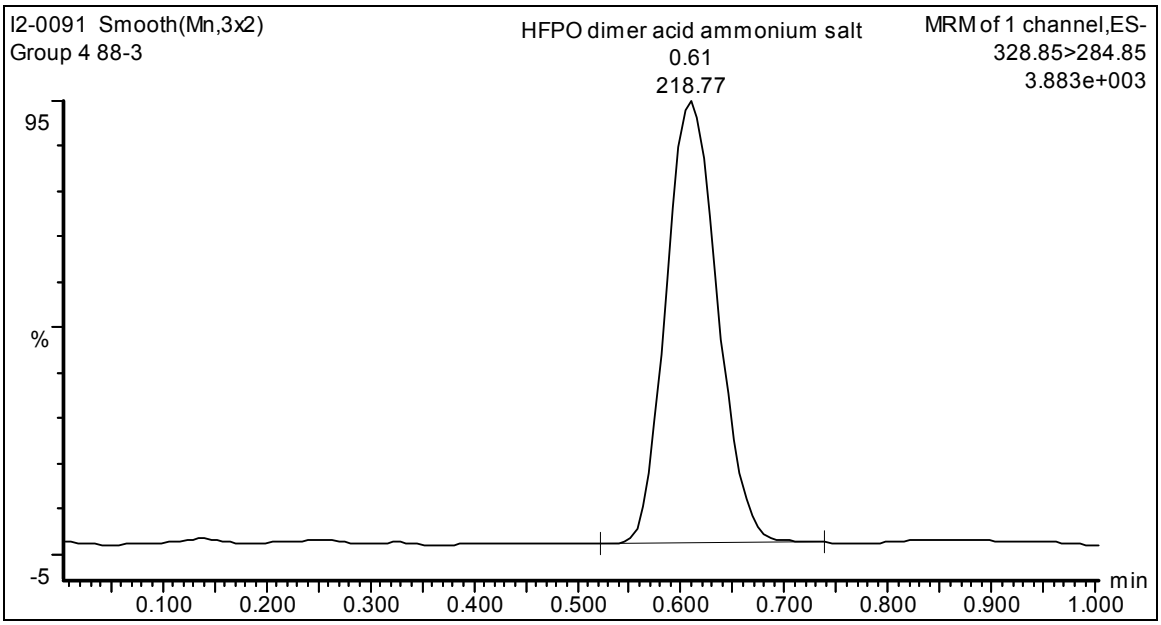

Figure 7: Representative Chromatogram Of A Processed 3 mg H-28397/mL Formulation Sample

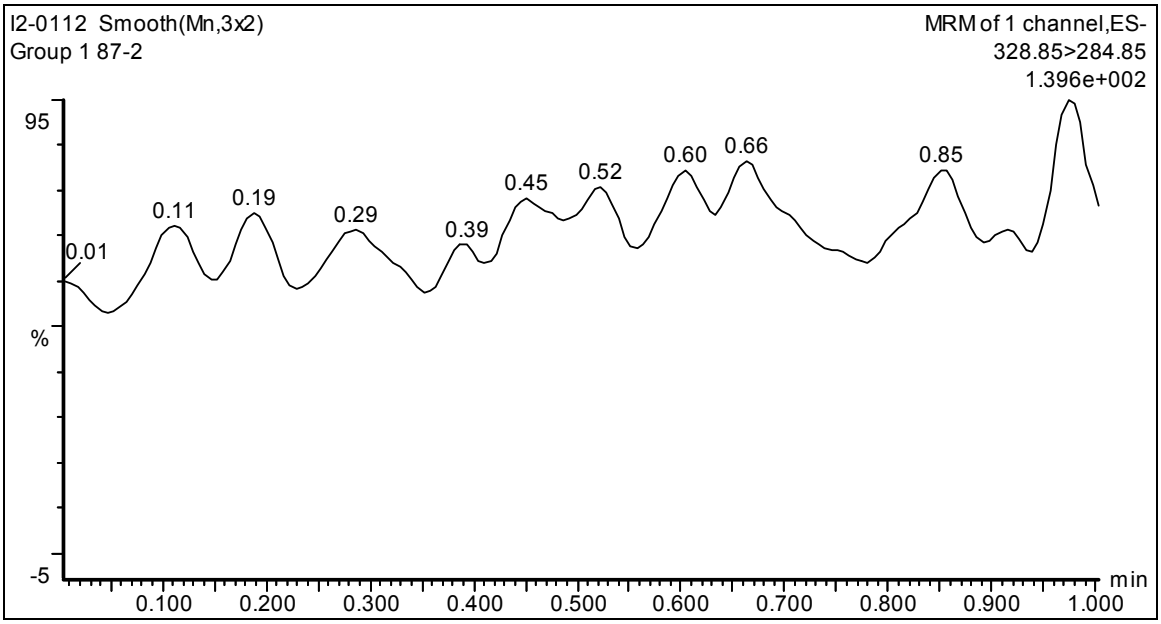

Figure 8: Representative Chromatogram Of A Processed Control Formulation Sample

A. Specificity/Selectivity

As shown in Figures 4 and 8 (and in contrast to the chromatograms shown in Figures 1 through 3 and 5 through 7, respectively), assay specificity/selectivity was confirmed when HPLC/MS/MS analysis of the control formulation samples revealed that there were no significant peaks at or near the retention time for the test article (approximately 0.5 to 0.6 minutes).

B. Assay Acceptability

In addition to the experimental samples, each analytical session consisted of (but was not limited to) calibration standards at 5 concentrations and triplicate QC samples at each of 3 concentrations. In this study, the formulations were prepared at theoretical test article concentrations of 0.01, 0.3 and 3 mg/mL and the QC samples were prepared at nominal test article concentrations of 0.00100, 0.0500, 0.500 and 50.0 mg/mL. For an analytical session to be considered valid, at least two-thirds of the QC samples with at least 1 at each concentration level had to be 85% to 115% of the QC target concentration. All reported results were from analytical sessions that met the acceptance criteria.

C. Assessment Of Test Article Homogeneity And Resuspension Homogeneity

Duplicate samples from the top, middle and bottom strata of formulations prepared at nominal test article concentrations of 0.01 and 3 mg H-28397/mL on 13-14 December 2007 were

analyzed to assess test article homogeneity. The formulations that remained after sampling were divided into aliquots representative of the volumes used for daily dispensation. Representative aliquots were stored at room temperature for 5 hours or 12 days refrigerated, at which times the test article in an aliquot was resuspended by stirring. Samples were collected from the top and bottom strata and analyzed to assess resuspension homogeneity. The results of the homogeneity and the 5-hour and 12-day resuspension homogeneity analyses are presented in Tables 1 through 3 with the overall statistics summarized in the following tables.

Homogeneity Assessment Of The 13-14 December 2007 Formulations

	Low Group (0.01 mg/mL)	High Group (3 mg/mL)
Mean Concentration (mg/mL)	0.0110	3.01
SD	0.00095	0.25
RSD (%)	8.6	8.3
Mean % of Target	110	100

The formulations analyzed met the WIL standard operating procedure (SOP) acceptance criteria for test article homogeneity, i.e., the RSD for the mean concentration was 10% or less at a concentration within the acceptable limits (85% to 115% of target concentration).

5-Hour Resuspension Homogeneity Assessment 13-14 December 2007 Formulations

	Low Group (0.01 mg/mL)	High Group (3 mg/mL)
Mean Concentration (mg/mL)	0.0102	2.92
SD	0.00038	0.31
RSD (%)	3.7	11
Mean % of Target	102	97.5

12-Day Resuspension Homogeneity Assessment 13-14 December 2007 Formulations

	Low Group (0.01 mg/mL)	High Group (3 mg/mL)
Mean Concentration (mg/mL)	0.0101	2.91
SD	0.00069	0.026
RSD (%)	6.9	0.91
Mean % of Target	101	96.9

The formulations stored at room-temperature for 5 hours and refrigerated 12 days met the WIL SOP acceptance criteria for resuspension homogeneity, i.e., the RSD for the mean

concentration was 10% or less, with the following exception. Following 5 hours of room temperature storage, the RSD for the mean concentration of the formulation prepared at 3 mg/mL was 11%. The out-of-specification result was believed to be due to drift in the ionization efficiency of the mass spectrometer. Consequently, the study director evaluated the data and considered it acceptable. To reduce the variability, the assay was cross-validated with modified diluent and mobile phases in another study (Haas, 2008) prior to the 12-day resuspension homogeneity assessment.

D. Test Article Stability In Aqueous Formulations

The formulations prepared on 13-14 December 2007 at target test article concentrations of 0.01 and 3 mg/mL were analyzed, stored at room-temperature for 5 hours or 12 days refrigerated and reanalyzed to assess test article stability. The stability results are presented in Tables 2 and 3 are summarized in the following table.

Time Point	Mean Concentration, mg/mL (% of Pre-Storage) Low Group (0.01 mg/mL)	High Group (3 mg/mL)
5 Hours (Room Temperature)	0.0102 (92.7)	2.92 (97.2)
12 Days (Refrigerated)	0.0101 (91.1)	2.91 (96.6)

The mean post-storage concentrations ranged from 91.1% to 97.2% of the pre-storage values, which met the previously stated WIL SOP requirement for stability.

E. Test Article Concentration In Aqueous Formulations

The results of the determination of test article concentration in formulations prepared prior to dosing and not used for dosing and in formulations used for dose administration are presented in Tables 4 through 7. The mean concentrations and percent of target values are summarized in the following table.

Date of Preparation	Group 1 (0 mg/mL)	Group 2 (Low) (0.01 mg/mL)	Group 3 (0.3 mg/mL)	Group 4 (High) (3 mg/mL)
13-14 December 2007*	NA	0.0112 (112)	NA	2.92(97.4)
17-18 December 2008	ND	0.00864(86.4)	0.233 (77.8)	3.26(109)
23 December 2008	ND	0.0108(108)	0.341 (114)	2.97(99.1)
7-8 January 2008	ND	0.0110(110)	0.306 (102)	2.99(99.7)

ND = No test article chromatographic peak detected
NA = Not Applicable
* = Prepared prior to dosing and not used for dosing

The analyzed formulations used for dose administration met the WIL SOP requirement for concentration acceptability for suspension formulations, i.e., the analyzed concentrations were 85% to 115% of the target concentrations with one exception. The Group 3 formulation prepared on 17-18 December 2007 at the target test article concentration of 0.3 mg/mL was 77.8% of target. Consequently, the study director changed the dose volume from 10 to 12 mL/kg for that Group 3 formulation. No test article was detected in the vehicle for administration to the control group (Group 1).

CONCLUSION

An HPLC/MS/MS method in the ESI- mode for the determination of H-28397 concentration in aqueous formulations was validated in a previous study (Haas, 2008). Aqueous formulations prepared at target test article concentrations of 0.01 and 3 mg/mL met the previously stated WIL SOP acceptance criteria for test article homogeneity, resuspension homogeneity and stability, with the following exception. The 13-14 December 2007 formulation prepared at the target test article concentration of 0.3 mg/mL and stored at room temperature for 5 hours failed to meet the WIL SOP requirement for resuspension homogeneity (11% RSD). The out-of-specification results were believed to be the result of drift in ionization efficiency in the mass spectrometer. Consequently, the study director evaluated the data and considered it acceptable. To reduce the variability, the assay was cross-validated with modified diluent and mobile phases in another study (Haas, 2008) prior to the 12-day resuspension homogeneity assessment.

The analyzed formulations used for dose administration met the WIL SOP requirement for concentration acceptability for suspension formulations with the following exception. The 17-18 December 2007 Group 3 formulation prepared at the target test article concentration of 0.3 mg/mL was 77.8% of H-28397. Consequently, the study director changed the dose volume from 10 to 12 mL/kg for that Group 3 formulation. No test article was detected in the vehicle for administration to the control group.

TABLES 1 - 7

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY

Table 1: Homogeneity Analysis Of The 13-14 December 2007 Formulations

(Analyzed 14-17 December 2007)

Dose Conc (mg/mL)	**Group/ Strata**	**Ref #** (189207 -)	**Run #**	**Analyzed Conc** (mg/mL)	**Percent of Target** (%)	**Mean Conc** (mg/mL)	**SD**	**RSD** (%)	**Mean Conc % of Target** (%)
0.01	Low/Top	9 - 1	I3-0023	0.0121	121	0.0110	0.00095	8.6	110
		9 - 2	I3-0024	0.00948	94.8				
	Low/Mid	9 - 3	I3-0025	0.0110	110				
		9 - 4	I3-0026	0.0114	114				
	Low/Btm	9 - 5	I3-0027	0.0118	118				
		9 - 6	I3-0028	0.0105	105				
3	High/Top	9 - 7	I3-0030	3.27	109	3.01	0.25	8.3	100
		9 - 8	I3-0031	3.17	106				
	High/Mid	9 - 9	I3-0032	2.90	96.8				
		9 - 10	I3-0033	2.94	97.9				
	High/Btm	9 - 11	I3-0034	2.59	86.3				
		9 - 12	I3-0035	3.18	106				

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A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY

Table 2: 5-Hour Resuspension Homogeneity And Room Temperature Storage Stability Analysis Of The 13-14 December 2007 Formulations (Combined Results)

(Analyzed 14-18 December 2007)

Dose Conc (mg/mL)	Group/ Strata	Ref # (189207 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Mean Conc % of Time Zero (%)
0.01	Low/Top	13 - 1	I3-0048	0.00979	97.9	0.0102	0.00038	3.7	102	92.7
		13 - 2	I3-0049	0.0106	106					
	Low/Btm	13 - 3	I3-0050	0.0105	105					
		13 - 4	I3-0051	0.0101	101					
3	High/Top	13 - 5	I3-0053	3.43	114	2.92	0.31	11	97.5	97.2
		* 26 - 1	I3-0165	2.92	97.2					
		13 - 6	I3-0054	2.66	88.6					
		* 26 - 2	I3-0166	3.26	109					
	High/Btm	13 - 7	I3-0055	2.45	81.6					
		* 26 - 3	I3-0167	2.89	96.4					
		13 - 8	I3-0056	2.96	98.6					
		* 26 - 4	I3-0168	2.84	94.6					

* Backup samples analyzed on 18 December 2007.

Time Zero Concentration

Group	Conc. (mg/mL)
Low	0.0110
High	3.01

189207 results.xls 5hrSTB(12-17-07) (2)
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A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY

Table 3: 12-Day Resuspension Homogeneity And Refrigerated Storage Stability Analysis Of The 13-14 December 2007 Formulations
(Analyzed 26-27 December 2007)

Dose Conc (mg/mL)	Group/ Strata	Ref # (189207 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)	Mean Conc % of Time Zero (%)
Collected and Processed 26Dec07; Secondary dilutions and analysis performed 27Dec07.										
0.01	Low/Top	39 - 1a	I3-0417	0.00920	92.0	0.0101	0.00069	6.9	101	91.1
		39 - 1a	I3-0434	0.00987	98.7					
		39 - 2a	I3-0413	0.0101	101					
		39 - 2a	I3-0435	0.00974	97.4					
		39 - 2a	I3-0348	0.00978	97.8					
	Low/Btm	39 - 3a	I3-0414	0.0111	111					
		39 - 3a	I3-0436	0.0101	101					
		39 - 3a	I3-0349	0.0110	110					
		39 - 4a	I3-0418	0.00910	91.0					
		39 - 4a	I3-0437	0.0107	107					
Collected, Processed and Analyzed 27Dec07.										
3	High/Top	39 - 5	I3-0353	2.87	95.8	2.91	0.026	0.91	96.9	96.6
		39 - 6	I3-0354	2.91	96.9					
	High/Btm	39 - 7	I3-0355	2.94	98.0					
		39 - 8	I3-0356	2.90	96.7					

Low Group formulations were analyzed twice on 27Dec07; a summary of the results are presented.

Time Zero Concentration

Group	Conc. (mg/mL)
Low	0.0110
High	3.01

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A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY

Table 4: Concentration Analysis Of The 13-14 December 2007 Formulations

(Analyzed 14-17 December 2007)

Dose Conc (mg/mL)	Group/ Strata	Ref # (189207 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
0.01	Low/Mid	9 - 3	I3-0025	0.0110	110	0.0112	0.00032	2.8	112
		9 - 4	I3-0026	0.0114	114				
3	High/Mid	9 - 9	I3-0032	2.90	96.8	2.92	0.024	0.83	97.4
		9 - 10	I3-0033	2.94	97.9				

189207 results.xls C(12-17-07)
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A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY

Table 5: Concentration Analysis Of The 17-18 December 2007 Formulations

(Analyzed 18 December 2007)

Dose Conc (mg/mL)	Group/ Strata	Ref # (189207 -)	Run #	Analyzed Conc (mg/mL)	Percent of Target (%)	Mean Conc (mg/mL)	SD	RSD (%)	Mean Conc % of Target (%)
0	1	22 - 1	I3-0223	. . .not detected . . .					
		22 - 2	I3-0224	. . .not detected . . .					
0.01	2	22 - 3	I3-0212	0.00889	88.9	0.00864	0.00035	4.0	86.4
		22 - 4	I3-0213	0.00840	84.0				
0.3	3	23 - 3	I3-0194	0.236	78.8	0.233	0.017	7.4	77.8
		23 - 4	I3-0195	0.230	76.6				
		23 - 7	I3-0225	0.240	80.1				
		23 - 8	I3-0226	0.214	71.2				
		23 - 9	I3-0227	0.219	72.9				
		23 - 10	I3-0228	0.262	87.2				
3	4	23 - 5	I3-0196	3.33	111	3.26	0.10	3.1	109
		23 - 6	I3-0197	3.19	106				

189207 results.xls C(12-18-07)
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A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY

Table 6: Concentration Analysis Of The 23 December 2007 Formulations

(Analyzed 26-27 December 2007)

Dose Conc (mg/mL)	**Group/ Strata**	**Ref #** (189207 -)	**Run #**	**Analyzed Conc** (mg/mL)	**Percent of Target** (%)	**Mean Conc** (mg/mL)	**SD**	**RSD** (%)	**Mean Conc % of Target** (%)
0	1	42 - 1	I3-0392	. . .not detected . . .					
		42 - 2	I3-0393	. . .not detected . . .					
0.01	2	42 - 3	I3-0369	0.0104	104	0.0108	0.00056	5.1	108
		42 - 4	I3-0370	0.0112	112				
0.3	3	43 - 1	I3-0371	0.353	118	0.341	0.017	4.9	114
		43 - 2	I3-0372	0.329	110				
3	4	43 - 3	I3-0373	2.94	98.0	2.97	0.047	1.6	99.1
		43 - 4	I3-0374	3.01	100				

Samples were collected and stored frozen on 23Dec07. Samples were removed from storage and processed on 26Dec07. Samples were analyzed 27Dec07.

189207 results.xls C(12-26-07)
Printed: 29May2008 11:20 AM

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY
OF H-28397 IN MICE WITH A 28-DAY RECOVERY

Table 7: Concentration Analysis Of The 7-8 January 2008 Formulations

(Analyzed 8 January 2008)

Dose Conc (mg/mL)	**Group/ Strata**	**Ref #** (189207 -)	**Run #**	**Analyzed Conc** (mg/mL)	**Percent of Target** (%)	**Mean Conc** (mg/mL)	**SD**	**RSD** (%)	**Mean Conc % of Target** (%)
0	1	87 - 1	I2-0111	. . .not detected . . .					
		87 - 2	I2-0112	. . .not detected . . .					
0.01	2	87 - 3	I2-0087	0.0105	105	0.0110	0.00067	6.1	110
		87 - 4	I2-0088	0.0114	114				
0.3	3	88 - 1	I2-0089	0.303	101	0.306	0.0039	1.3	102
		88 - 2	I2-0090	0.309	103				
3	4	88 - 3	I2-0091	3.12	104	2.99	0.19	6.2	99.7
		88 - 4	I2-0092	2.86	95.3				

189207 results.xls C(1-8-08)
Printed: 29May2008 11:20 AM

ATTACHMENT I

Supporting Data

Quantify Compound Summary Report **MassLynx 4.1**

Dataset: Z:\189207.PRO\I3-189207a1.qld

Last Altered: Thursday, December 20, 2007 09:32:32 Eastern Standard Time
Printed: At Thursday, December 20, 2007 09:35:17 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Method: Z:\189207.pro\MethDB\189207.mdb 17 Dec 2007 20:49:05
Calibration: 20 Dec 2007 09:32:31

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
1	I3-0001	3-9	sys suit		0.50	8454	8454	bb	1.00	0.0005448	
2	I3-0002	3-9	sys suit		0.50	8032	8032	bb	1.00	0.0005062	
3	I3-0003	3-9	sys suit		0.51	8356	8356	bb	1.00	0.0005357	
4	I3-0004	3-9	sys suit		0.51	7777	7777	bb	1.00	0.0004833	
5	I3-0005	3-9	sys suit		0.51	7852	7852	bb	1.00	0.0004900	
6	I3-0006	3-9	sys suit		0.51	8993	8993	bb	1.00	0.0005952	
7	I3-0007	3-9	sys suit		0.51	8492	8492	bb	1.00	0.0005483	
8	I3-0008	3-9	sys suit		0.50	7706	7706	bb	1.00	0.0004770	
9	I3-0009	3-9	sys suit		0.50	8042	8042	bb	1.00	0.0005071	
10	I3-0010		DI Water		0.51	233.4	233.4	bb	1.00	0.000003522	
11	I3-0011	3-1	STD 100ng	0.000100	0.50	2649	2649	bb	1.00	0.0001043	4.27
12	I3-0012	3-4	STD 250ng	0.000250	0.51	5496	5496	bb	1.00	0.0002942	17.7
13	I3-0013	3-7	STD 500ng	0.000500	0.50	8747	8747	bb	1.00	0.0005720	14.4
14	I3-0014	3-10	STD 750ng	0.000750	0.51	10420	10420	bb	1.00	0.0007355	-1.93
15	I3-0015	3-13	STD 1000ng	0.00100	0.51	13330	13330	bb	1.00	0.001048	4.83
16	I3-0016		DI Water		0.51	248.6	248.6	bb	1.00	0.000003840	
17	I3-0017	6-1	blank		0.50	227.3	227.3	bb	2.00	0.000006791	
18	I3-0018	6-2	QC .001mg	0.00100	0.51	8014	8014	bb	2.00	0.001009	0.914
19	I3-0019	6-5	QC .05 mg	0.0500	0.50	7773	7773	bb	100	0.04830	-3.41
20	I3-0020	7-1	QC .5 mg	0.500	0.50	8852	8852	bb	1000	0.5819	16.4
21	I3-0021	7-4	QC 50 mg	50.0	0.50	8333	8333	bb	100000	53.36	6.73
22	I3-0022		DI Water		0.50	268.2	268.2	bb	1.00	0.000004264	
23	I3-0023	9-1	Low, 0.01 mg/mL		0.51	9077	9077	bb	20.0	0.01207	20.7
24	I3-0024	9-2	Low, 0.01 mg/mL		0.51	7674	7674	bb	20.0	0.009484	-5.16
25	I3-0025	9-3	Low, 0.01 mg/mL		0.51	8490	8490	bb	20.0	0.01096	9.62
26	I3-0026	9-4	Low, 0.01 mg/mL		0.50	8731	8731	bb	20.0	0.01141	14.1
27	I3-0027	9-5	Low, 0.01 mg/mL		0.51	8961	8961	bb	20.0	0.01184	18.4
28	I3-0028	9-6	Low, 0.01 mg/mL		0.50	8256	8256	bb	20.0	0.01053	5.32
29	I3-0029		DI Water		0.50	219.4	219.4	bb	1.00	0.000003234	
30	I3-0030	9-7	High, 30 mg/mL		0.50	8460	8460	bb	6000	3.272	9.06
31	I3-0031	9-8	High, 30 mg/mL		0.51	8267	8267	bb	6000	3.165	5.50
32	I3-0032	9-9	High, 30 mg/mL		0.51	7784	7784	bb	6000	2.904	-3.21
33	I3-0033	9-10	High, 30 mg/mL		0.50	7848	7848	bb	6000	2.938	-2.07

Quantify Compound Summary Report MassLynx 4.1

Dataset: Z:\189207.PRO\I3-189207a1.qld

Last Altered: Thursday, December 20, 2007 09:32:32 Eastern Standard Time
Printed: At Thursday, December 20, 2007 09:35:17 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
34	I3-0034	9-11	High, 30 mg/mL		0.50	7186	7186	bb	6000	2.590	-13.7
35	I3-0035	9-12	High, 30 mg/mL		0.50	8288	8288	bb	6000	3.177	5.89
36	I3-0036		DI Water		0.51	254.9	254.9	bb	1.00	0.000003975	
37	I3-0037	6-3	QC .001mg	0.00100	0.51	7162	7162	bb	2.00	0.0008590	-14.1
38	I3-0038	6-6	QC .05 mg	0.0500	0.50	7538	7538	bb	100	0.04622	-7.56
39	I3-0039	7-2	QC .5 mg	0.500	0.51	8536	8536	bb	1000	0.5523	10.5
40	I3-0040	7-5	QC 50 mg	50.0	0.50	8637	8637	bb	100000	56.18	12.4
41	I3-0041		DI Water		0.50	281.7	281.7	bb	1.00	0.000004562	
42	I3-0042	3-2	STD 100ng	0.000100	0.51	2642	2642	bb	1.00	0.0001039	3.90
43	I3-0043	3-5	STD 250ng	0.000250	0.51	5217	5217	bb	1.00	0.0002731	9.24
44	I3-0044	3-8	STD 500ng	0.000500	0.50	8370	8370	bb	1.00	0.0005370	7.39
45	I3-0045	3-11	STD 750ng	0.000750	0.51	11000	11000	bb	1.00	0.0007947	5.96
46	I3-0046	3-14	STD 1000ng	0.00100	0.51	13980	13980	bb	1.00	0.001123	12.3
47	I3-0047		DI Water		0.50	253.3	253.3	bb	1.00	0.000003941	
48	I3-0048	13-1	Low, 0.01 mg/mL		0.51	7846	7846	bb	20.0	0.009790	-2.10
49	I3-0049	13-2	Low, 0.01 mg/mL		0.50	8284	8284	bb	20.0	0.01058	5.83
50	I3-0050	13-3	Low, 0.01 mg/mL		0.51	8254	8254	bb	20.0	0.01053	5.28
51	I3-0051	13-4	Low, 0.01 mg/mL		0.50	8006	8006	bb	20.0	0.01008	0.774
52	I3-0052		DI Water		0.50	271.9	271.9	bb	1.00	0.000004343	
53	I3-0053	13-5	High, 30 mg/mL		0.51	8735	8735	bb	6000	3.426	14.2
54	I3-0054	13-6	High, 30 mg/mL		0.51	7321	7321	bb	6000	2.659	-11.4
55	I3-0055	13-7	High, 30 mg/mL		0.50	6908	6908	bb	6000	2.447	-18.4
56	I3-0056	13-8	High, 30 mg/mL		0.50	7884	7884	bb	6000	2.957	-1.43
57	I3-0057		DI Water		0.50	257.6	257.6	bb	1.00	0.000004034	
58	I3-0058	189205-...	Low, 0.03 mg/mL		0.51	7812	7812	bb	60.0	0.02918	-2.72
59	I3-0059	189205-...	Low, 0.03 mg/mL		0.50	6237	6237	bb	60.0	0.02115	-29.5
60	I3-0060	189205-...	Low, 0.03 mg/mL		0.50	8657	8657	bb	60.0	0.03382	12.7
61	I3-0061	189205-...	Low, 0.03 mg/mL		0.51	6830	6830	bb	60.0	0.02408	-19.7
62	I3-0062		DI Water		0.50	261.0	261.0	bb	1.00	0.000004107	
63	I3-0063	6-4	QC .001mg	0.00100	0.50	6949	6949	bb	2.00	0.0008227	-17.7
64	I3-0064	6-7	QC .05 mg	0.0500	0.51	6075	6075	bb	100	0.03394	-32.1
65	I3-0065	7-3	QC .5 mg	0.500	0.50	8146	8146	bb	1000	0.5166	3.31
66	I3-0066	7-6	QC 50 mg	50.0	0.51	8276	8276	bb	100000	52.84	5.67
67	I3-0067		DI Water		0.50	235.7	235.7	bb	1.00	0.000003568	
68	I3-0068	3-3	STD 100ng	0.000100	0.51	2381	2381	bb	1.00	0.00008968	-10.3
69	I3-0069	3-6	STD 250ng	0.000250	0.51	4441	4441	bb	1.00	0.0002171	-13.2

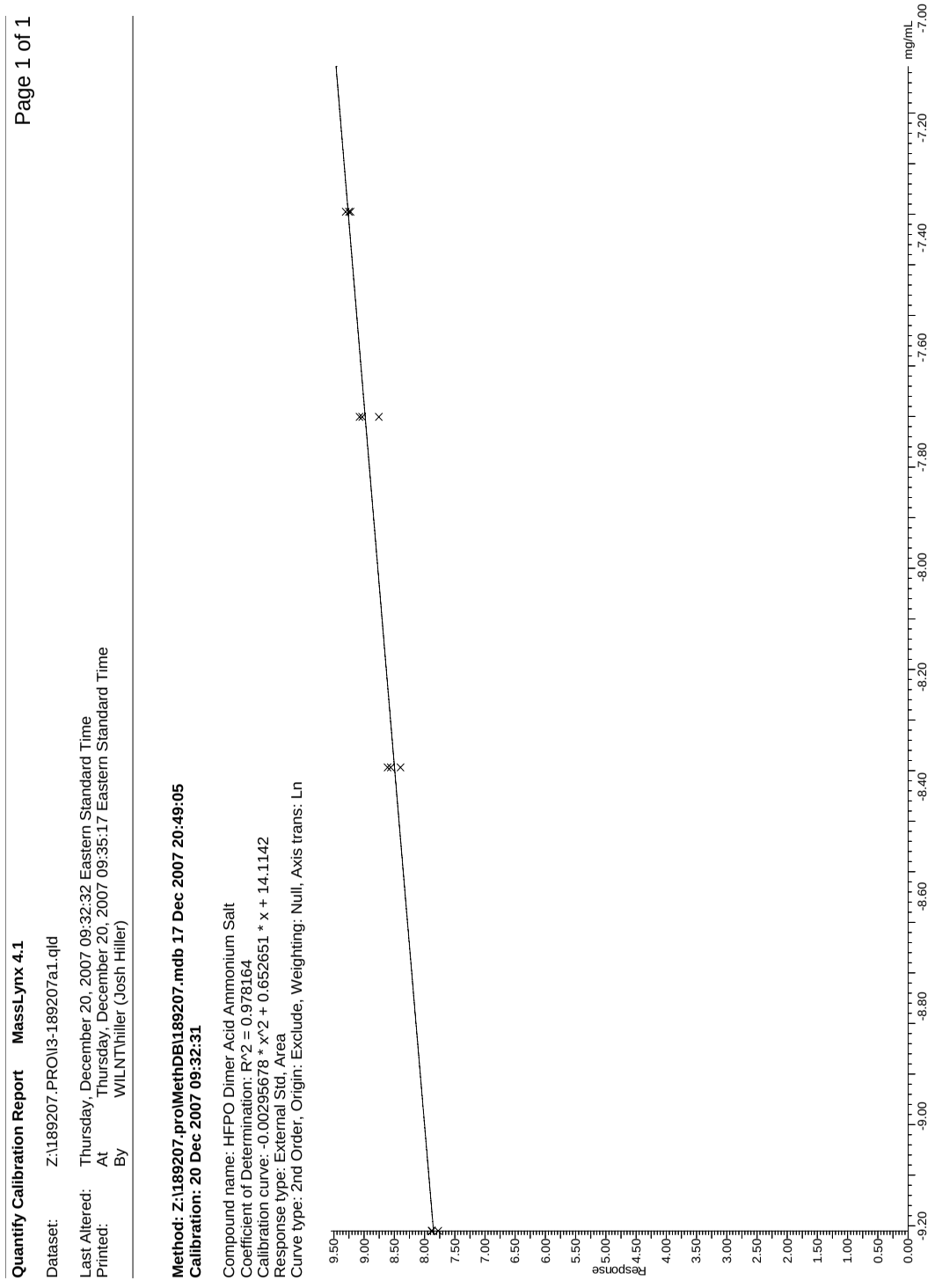
Quantify Compound Summary Report **MassLynx 4.1**

Dataset: Z:\189207.PRO\I3-189207a1.qld

Last Altered: Thursday, December 20, 2007 09:32:32 Eastern Standard Time
Printed: At Thursday, December 20, 2007 09:35:17 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
70	I3-0070	3-9	STD 500ng	0.000500	0.51	6363	6363	bb	1.00	0.0003626	-27.5
71	I3-0071	3-12	STD 750ng	0.000750	0.50	10140	10140	bb	1.00	0.0007077	-5.64
72	I3-0072	3-15	STD 1000ng	0.00100	0.50	11960	11960	bb	1.00	0.0008970	-10.3
73	I3-0073		DI Water		0.50	254.0	254.0	bb	1.00	0.000003956	


Quantify Calibration Report MassLynx 4.1
Page 1 of 1
Dataset: Z:\189207.PRO\3-189207a1.qld
Last Altered: Thursday, December 20, 2007 09:32:32 Eastern Standard Time
Printed: At Thursday, December 20, 2007 09:35:17 Eastern Standard Time
By WILNT\hiller (Josh Hiller)
Method: Z:\189207.proIMethDB\189207.mdb 17 Dec 2007 20:49:05
Calibration: 20 Dec 2007 09:32:31
Compound name: HFPO Dimer Acid Ammonium Salt
Coefficient of Determination: R^2 = 0.978164
Calibration curve: -0.00295678 * x^2 + 0.652651 * x + 14.1142
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: Null, Axis trans: Ln
Response
mg/mL

Dataset: C:\MassLynx\189207.PRO\I3-189207b.qld

Last Altered: Thursday, December 20, 2007 09:44:28 Eastern Standard Time
Printed: At Monday, January 07, 2008 11:03:47 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Method: Z:\189207.pro\MethDB\189207.mdb 17 Dec 2007 20:49:05
Calibration: 20 Dec 2007 09:44:28

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
1	I3-0143	17-7	STD 500ng		0.51	7831.500		7831.50000	bb	1.000	0.0005	
2	I3-0144	17-7	STD 500ng		0.51	7396.205		7396.20500	bb	1.000	0.0005	
3	I3-0145	17-7	STD 500ng		0.50	7705.550		7705.55000	bb	1.000	0.0005	
4	I3-0146	17-7	STD 500ng		0.50	7364.435		7364.43500	bb	1.000	0.0005	
5	I3-0147	17-7	STD 500ng		0.51	7850.402		7850.40200	bb	1.000	0.0005	
6	I3-0148	17-7	STD 500ng		0.50	7481.581		7481.58100	bb	1.000	0.0005	
7	I3-0149	17-7	STD 500ng		0.50	7525.181		7525.18100	bb	1.000	0.0005	
8	I3-0150	17-7	STD 500ng		0.51	7601.481		7601.48100	bb	1.000	0.0005	
9	I3-0151	17-7	STD 500ng		0.50	7114.625		7114.62500	bb	1.000	0.0005	
10	I3-0152		DI Water							1.000		
11	I3-0153	17-1	STD 100ng	0.00	0.51	2115.850		2115.85000	bb	1.000	0.0001	-1.743
12	I3-0154	17-4	STD 250ng	0.00	0.50	4954.264		4954.26400	bb	1.000	0.0003	17.712
13	I3-0155	17-7	STD 500ng	0.00	0.50	7027.122		7027.12200	bb	1.000	0.0005	-6.470
14	I3-0156	17-10	STD 750ng	0.00	0.50	11464.348		11464.34800	bb	1.000	0.0009	20.871
15	I3-0157	17-13	STD 1000ng	0.00	0.50	13111.881		13111.88100	bb	1.000	0.0011	9.013
16	I3-0158		DI Water		0.51	14.444		14.44400	bb	1.000	0.0000	
17	I3-0159	20-1	blank		0.50	5548.580		5548.58000	bb	2.000	0.0007	
18	I3-0160	20-2	QC .001mg	0.00	0.51	7483.615		7483.61500	bb	2.000	0.0010	1.750

Dataset: C:\MassLynx\189207.PRO\I3-189207b.qld

Last Altered: Thursday, December 20, 2007 09:44:28 Eastern Standard Time
Printed: At Monday, January 07, 2008 11:03:47 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
19	I3-0161	20-5	QC .05 mg	0.05	0.50	7604.846		7604.84600	bb	100.0...	0.0520	3.966
20	I3-0162	21-1	QC .5 mg	0.50	0.51	8329.763		8329.76300	bb	1000....	0.5875	17.502
21	I3-0163	21-4	QC 50 mg	50.00	0.51	6466.751		6466.75100	bb	1000...	41.8594	-16.281
22	I3-0164		DI Water		0.51	19.378		19.37800	bb	1.000	0.0000	
23	I3-0165	26-1	High, bckups, 3.00mg/...		0.50	7234.393		7234.39300	bb	6000....	2.9171	-2.762
24	I3-0166	26-2	High, bckups, 3.00mg/...		0.50	7852.074		7852.07400	bb	6000....	3.2558	8.528
25	I3-0167	26-3	High, bckups, 3.00mg/...		0.51	7190.354		7190.35400	bb	6000....	2.8934	-3.554
26	I3-0168	26-4	High, bckups, 3.00mg/...		0.50	7089.119		7089.11900	db	6000....	2.8390	-5.365
27	I3-0169		DI Water		0.51	15.289		15.28900	bb	1.000	0.0000	
28	I3-0170		DI Water		0.50	11.853		11.85300	bb	1.000	0.0000	
29	I3-0171	18920...	High, 30 mg/mL		0.50	7443.589		7443.58900	bb	6000...	30.3063	1.021
30	I3-0172	18920...	High, 30 mg/mL		0.51	6582.499		6582.49900	bb	6000...	25.7160	-14.280
31	I3-0173	18920...	High, 30 mg/mL		0.50	7518.217		7518.21700	bb	6000...	30.7142	2.381
32	I3-0174	18920...	High, 30 mg/mL		0.50	7645.213		7645.21300	bb	6000...	31.4120	4.707
33	I3-0175		DI Water		0.51	22.688		22.68800	bb	1.000	0.0000	
34	I3-0176	20-3	QC .001mg	0.00	0.50	6539.204		6539.20400	bb	2.000	0.0008	-15.030
35	I3-0177	20-6	QC .05 mg	0.05	0.50	8204.299		8204.29900	bb	100.0...	0.0576	15.125
36	I3-0178	21-2	QC .5 mg	0.50	0.50	7789.451		7789.45100	bb	1000....	0.5368	7.367
37	I3-0179	21-5	QC 50 mg	50.00	0.50	6760.524		6760.52400	bb	1000...	44.4119	-11.176
38	I3-0180		DI Water		0.50	10.767		10.76700	bb	1.000	0.0000	
39	I3-0181	17-2	STD 100ng	0.00	0.51	2139.205		2139.20500	bb	1.000	0.0001	-0.368
40	I3-0182	17-5	STD 250ng	0.00	0.51	4072.667		4072.66700	bb	1.000	0.0002	-8.908

Quantify Compound Summary Report **MassLynx 4.1** Page 3 of 5

Dataset: C:\MassLynx\189207.PRO\I3-189207b.qld

Last Altered: Thursday, December 20, 2007 09:44:28 Eastern Standard Time
Printed: At Monday, January 07, 2008 11:03:47 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
41	I3-0183	17-8	STD 500ng	0.00	0.50	6747.403		6747.40300	bb	1.000	0.0004	-11.406
42	I3-0184	17-11	STD 750ng	0.00	0.51	10582.507		10582.50700	bb	1.000	0.0008	8.353
43	I3-0185	17-14	STD 1000ng	0.00	0.51	10510.114		10510.11400	bb	1.000	0.0008	-19.492
44	I3-0186		DI Water		0.51	24.399		24.39900	bb	1.000	0.0000	
45	I3-0187	18920...	Low, 0.03 mg/mL		0.50	7746.476		7746.47600	bb	60.000	0.0320	6.572
46	I3-0188	18920...	Low, 0.03 mg/mL		0.51	8311.007		8311.00700	bb	60.000	0.0351	17.146
47	I3-0189	18920...	Low, 0.03 mg/mL		0.51	8039.894		8039.89400	bb	60.000	0.0336	12.031
48	I3-0190	18920...	Low, 0.03 mg/mL		0.50	8191.164		8191.16400	bb	60.000	0.0345	14.877
49	I3-0191		DI Water		0.50	23.579		23.57900	bb	1.000	0.0000	
50	I3-0192	23-1	Grp 2, .1mg/mL		0.50	1258.980		1258.98000	bb	200.0...	0.0103	2.623
51	I3-0193	23-2	Grp 2, .1mg/mL		0.50	1274.351		1274.35100	bb	200.0...	0.0104	4.177
52	I3-0194	23-3	Grp 3, 0.3 mg/mL		0.50	6178.526		6178.52600	bb	600.0...	0.2364	-21.205
53	I3-0195	23-4	Grp 3, 0.3 mg/mL		0.50	6048.675		6048.67500	bb	600.0...	0.2298	-23.396
54	I3-0196	23-5	Grp 4, 3.0 mg/mL		0.50	7987.051		7987.05100	bb	6000....	3.3313	11.042
55	I3-0197	23-6	Grp 4, 3.0 mg/mL		0.50	7730.253		7730.25300	bb	6000....	3.1882	6.273
56	I3-0198		DI Water		0.51	22.721		22.72100	bb	1.000	0.0000	
57	I3-0199	20-4	QC .001mg	0.00	0.51	6650.086		6650.08600	bb	2.000	0.0009	-13.105
58	I3-0200	20-7	QC .05 mg	0.05	0.50	7431.879		7431.87900	bb	100.0...	0.0504	0.808
59	I3-0201	21-3	QC .5 mg	0.50	0.50	7298.219		7298.21900	bb	1000....	0.4919	-1.613
60	I3-0202	21-6	QC 50 mg	50.00	0.50	8316.497		8316.49700	bb	1000...	58.6251	17.250
61	I3-0203		DI Water		0.50	19.909		19.90900	bb	1.000	0.0000	
62	I3-0204	17-3	STD 100ng	0.00	0.50	2251.254		2251.25400	bb	1.000	0.0001	6.291

Quantify Compound Summary Report **MassLynx 4.1**

Dataset: C:\MassLynx\189207.PRO\I3-189207b.qld

Last Altered: Thursday, December 20, 2007 09:44:28 Eastern Standard Time
Printed: At Monday, January 07, 2008 11:03:47 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
63	I3-0205	17-6	STD 250ng	0.00	0.50	3818.057		3818.05700	bb	1.000	0.0002	-16.244
64	I3-0206	17-9	STD 500ng	0.00	0.51	7953.942		7953.94200	bb	1.000	0.0006	10.424
65	I3-0207	17-12	STD 750ng	0.00	0.50	11167.385		11167.38500	bb	1.000	0.0009	16.609
66	I3-0208	17-15	STD 1000ng	0.00	0.51	11183.623		11183.62300	bb	1.000	0.0009	-12.369
67	I3-0209		DI Water		0.51	20.351		20.35100	bb	1.000	0.0000	
68	I3-0210		DI Water		0.51	17.224		17.22400	bb	1.000	0.0000	
69	I3-0211											
70	I3-0212	22-3	Grp 2, .01mg/mL		0.50	3997.349		3997.34900	bb	40.000	0.0089	-11.096
71	I3-0213	22-4	Grp 2, .01mg/mL		0.50	3825.244		3825.24400	bb	40.000	0.0084	-16.040
72	I3-0214		DI Water		0.50	19.437		19.43700	bb	1.000	0.0000	
73	I3-0215		DI Water		0.50	17.462		17.46200	bb	1.000	0.0000	
74	I3-0216		DI Water		0.50	18.286		18.28600	bb	1.000	0.0000	
75	I3-0217		DI Water		0.50	20.740		20.74000	bb	1.000	0.0000	
76	I3-0218		DI Water		0.50	20.518		20.51800	bb	1.000	0.0000	
77	I3-0219		DI Water		0.50	20.965		20.96500	bb	1.000	0.0000	
78	I3-0220		DI Water		0.50	23.152		23.15200	bb	1.000	0.0000	
79	I3-0221		DI Water		0.50	26.274		26.27400	bb	1.000	0.0000	
80	I3-0222		DI Water		0.50	46.341		46.34100	bb	1.000	0.0000	
81	I3-0223	22-1	Grp 1, blank							1.000		
82	I3-0224	22-2	Grp 1, blank		0.50	12.653		12.65300	bb	1.000	0.0000	
83	I3-0225	23-7	Grp 3, 0.3 mg/mL		0.51	6257.873		6257.87300	bb	600.0...	0.2404	-19.858
84	I3-0226	23-8	Grp 3, 0.3 mg/mL		0.50	5722.473		5722.47300	bb	600.0...	0.2135	-28.821

Dataset: C:\MassLynx\189207.PRO\I3-189207b.qld

Last Altered: Thursday, December 20, 2007 09:44:28 Eastern Standard Time
Printed: At Monday, January 07, 2008 11:03:47 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
85	I3-0227	23-9	Grp 3, 0.3 mg/mL		0.50	5827.888		5827.88800	bb	600.0...	0.2188	-27.080
86	I3-0228	23-10	Grp 3, 0.3 mg/mL		0.50	6668.684		6668.68400	bb	600.0...	0.2617	-12.781

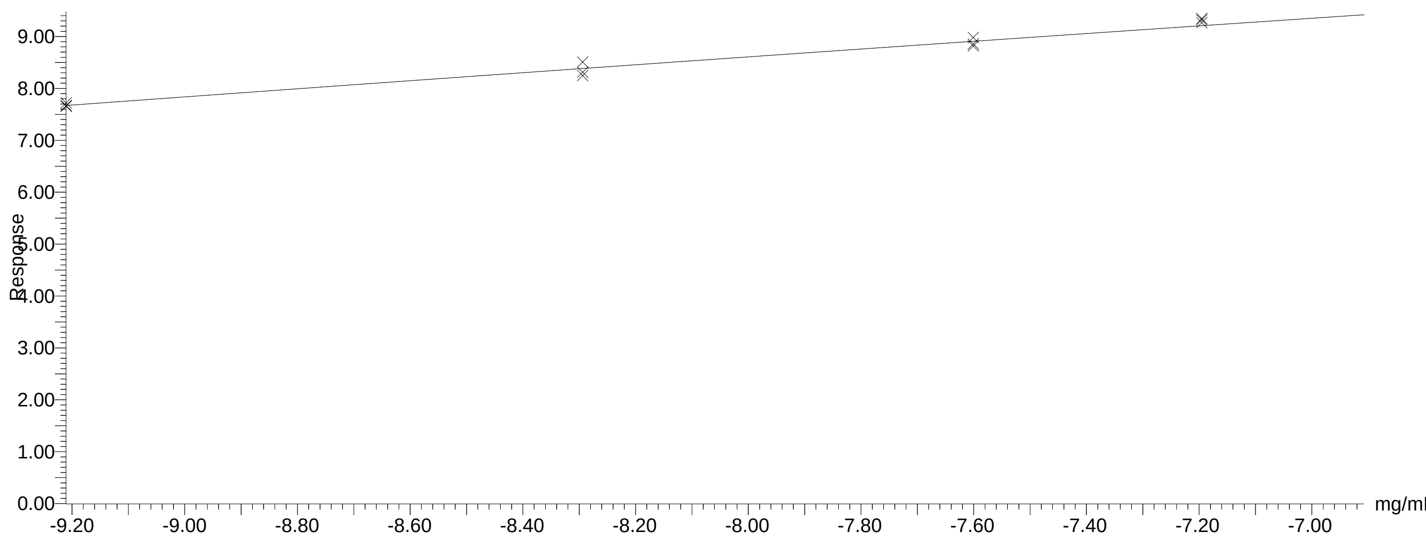
Quantify Calibration Report **MassLynx 4.1**

Dataset: C:\MassLynx\189207.PRO\I3-189207b.qld

Last Altered: Thursday, December 20, 2007 09:44:28 Eastern Standard Time
Printed: At Monday, January 07, 2008 11:03:47 Eastern Standard Time
By WILNT\hiller (Josh Hiller)

Method: Z:\189207.pro\MethDB\189207.mdb 17 Dec 2007 20:49:05
Calibration: 20 Dec 2007 09:44:28

Compound name: HFPO Dimer Acid Ammonium Salt
Coefficient of Determination: R^2 = 0.978264
Calibration curve: -0.0134522 * x^2 + 0.542086 * x + 13.8051
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: Null, Axis trans: Ln



Quantify Compound Summary Report **MassLynx 4.1**

Dataset: Untitled

Last Altered: Tuesday, January 08, 2008 16:29:22 Eastern Standard Time
Printed: At Tuesday, January 08, 2008 16:29:57 Eastern Standard Time
By WILNT\acgenuser (acgenuser)

Method: \\Lcms02\LCMS02\189207.PRO\MethDB\I2-189207a.mdb 08 Jan 2008 14:34:39
Calibration: 08 Jan 2008 16:29:22

Compound name: HFPO dimer acid ammonium salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
1	I2-0070	82-7	sys suit		0.61	231.1	231.1	bb	1.00	0.0005611	
2	I2-0071	82-7	sys suit		0.61	235.0	235.0	bb	1.00	0.0005744	
3	I2-0072	82-7	sys suit		0.60	232.4	232.4	bb	1.00	0.0005655	
4	I2-0073	82-7	sys suit		0.60	225.3	225.3	bb	1.00	0.0005417	
5	I2-0074	82-7	sys suit		0.61	223.6	223.6	bb	1.00	0.0005360	
6	I2-0075		Diluent						1.00		
7	I2-0076	82-1	STD 100ng	0.000100	0.61	57.08	57.08	bb	1.00	0.00009285	-7.15
8	I2-0077	82-4	STD 250ng	0.000250	0.60	132.9	132.9	bb	1.00	0.0002668	6.71
9	I2-0078	82-7	STD 500ng	0.000500	0.61	212.4	212.4	bb	1.00	0.0004994	-0.110
10	I2-0079	82-10	STD 750ng	0.000750	0.61	298.0	298.0	bb	1.00	0.0008027	7.03
11	I2-0080	82-13	STD 1000ng	0.00100	0.60	359.9	359.9	bb	1.00	0.001055	5.53
12	I2-0081		Diluent						1.00		
13	I2-0082	85-2	QC .001mg	0.00100	0.61	209.7	209.7	bb	2.00	0.0009815	-1.85
14	I2-0083	85-5	QC .05 mg	0.0500	0.61	220.8	220.8	bb	100	0.05269	5.37
15	I2-0084	86-1	QC .5 mg	0.500	0.61	218.5	218.5	bb	1000	0.5194	3.88
16	I2-0085	86-4	QC 50 mg	50.0	0.61	215.8	215.8	bb	100000	51.05	2.10
17	I2-0086		Diluent						1.00		
18	I2-0087	87-3	Group 2		0.61	219.9	219.9	bb	20.0	0.01048	4.80
19	I2-0088	87-4	Group 2		0.61	234.1	234.1	bb	20.0	0.01142	14.2

Quantify Compound Summary Report   MassLynx 4.1

Dataset: Untitled

Last Altered: Tuesday, January 08, 2008 16:29:22 Eastern Standard Time
Printed: At Tuesday, January 08, 2008 16:29:57 Eastern Standard Time
By WILNT\acgenuser (acgenuser)

Compound name: HFPO dimer acid ammonium salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
20	I2-0089	88-1	Group 3		0.61	214.2	214.2	bb	600	0.3033	1.09
21	I2-0090	88-2	Group 3		0.61	217.1	217.1	bb	600	0.3088	2.94
22	I2-0091	88-3	Group 4		0.61	218.8	218.8	bb	6000	3.121	4.04
23	I2-0092	88-4	Group 4		0.61	205.2	205.2	bb	6000	2.858	-4.72
24	I2-0093		Diluent						1.00		
25	I2-0094	85-3	QC .001mg	0.00100	0.61	207.9	207.9	bb	2.00	0.0009701	-2.99
26	I2-0095	85-6	QC .05 mg	0.0500	0.61	227.5	227.5	bb	100	0.05490	9.79
27	I2-0096	86-2	QC .5 mg	0.500	0.61	216.3	216.3	bb	1000	0.5120	2.40
28	I2-0097	86-5	QC 50 mg	50.0	0.61	202.8	202.8	bb	100000	46.88	-6.24
29	I2-0098		Diluent						1.00		
30	I2-0099	82-2	STD 100ng	0.000100	0.60	64.43	64.43	bb	1.00	0.0001075	7.46
31	I2-0100	82-5	STD 250ng	0.000250	0.61	122.3	122.3	bb	1.00	0.0002395	-4.19
32	I2-0101	82-8	STD 500ng	0.000500	0.61	213.2	213.2	bb	1.00	0.0005021	0.425
33	I2-0102	82-11	STD 750ng	0.000750	0.61	283.6	283.6	bb	1.00	0.0007480	-0.264
34	I2-0103	82-14	STD 1000ng	0.00100	0.61	341.8	341.8	bb	1.00	0.0009784	-2.16
35	I2-0104		Diluent						1.00		
36	I2-0105		Diluent						1.00		
37	I2-0106		Diluent						1.00		
38	I2-0107		Diluent						1.00		
39	I2-0108		Diluent						1.00		
40	I2-0109		Diluent						1.00		
41	I2-0110	85-1	blank						2.00		

Quantify Compound Summary Report **MassLynx 4.1**

Dataset: Untitled

Last Altered: Tuesday, January 08, 2008 16:29:22 Eastern Standard Time
Printed: At Tuesday, January 08, 2008 16:29:57 Eastern Standard Time
By WILNT\acgenuser (acgenuser)

Compound name: HFPO dimer acid ammonium salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
42	I2-0111	87-1	Group 1						1.00		
43	I2-0112	87-2	Group 1						1.00		
44	I2-0113		Diluent						1.00		
45	I2-0114	85-4	QC .001mg	0.00100	0.61	214.1	214.1	bb	2.00	0.001010	1.00
46	I2-0115	85-7	QC .05 mg	0.0500	0.61	222.7	222.7	bb	100	0.05333	6.66
47	I2-0116	86-3	QC .5 mg	0.500	0.61	210.0	210.0	bb	1000	0.4917	-1.66
48	I2-0117	86-6	QC 50 mg	50.0	0.60	197.6	197.6	bb	100000	45.26	-9.47
49	I2-0118		Diluent						1.00		
50	I2-0119	82-3	STD 100ng	0.000100	0.60	60.80	60.80	bb	1.00	0.0001002	0.182
51	I2-0120	82-6	STD 250ng	0.000250	0.60	124.3	124.3	bb	1.00	0.0002447	-2.14
52	I2-0121	82-9	STD 500ng	0.000500	0.60	214.3	214.3	bb	1.00	0.0005056	1.11
53	I2-0122	82-12	STD 750ng	0.000750	0.61	266.0	266.0	bb	1.00	0.0006830	-8.93
54	I2-0123	82-15	STD 1000ng	0.00100	0.60	343.0	343.0	bb	1.00	0.0009836	-1.64
55	I2-0124		Diluent						1.00		

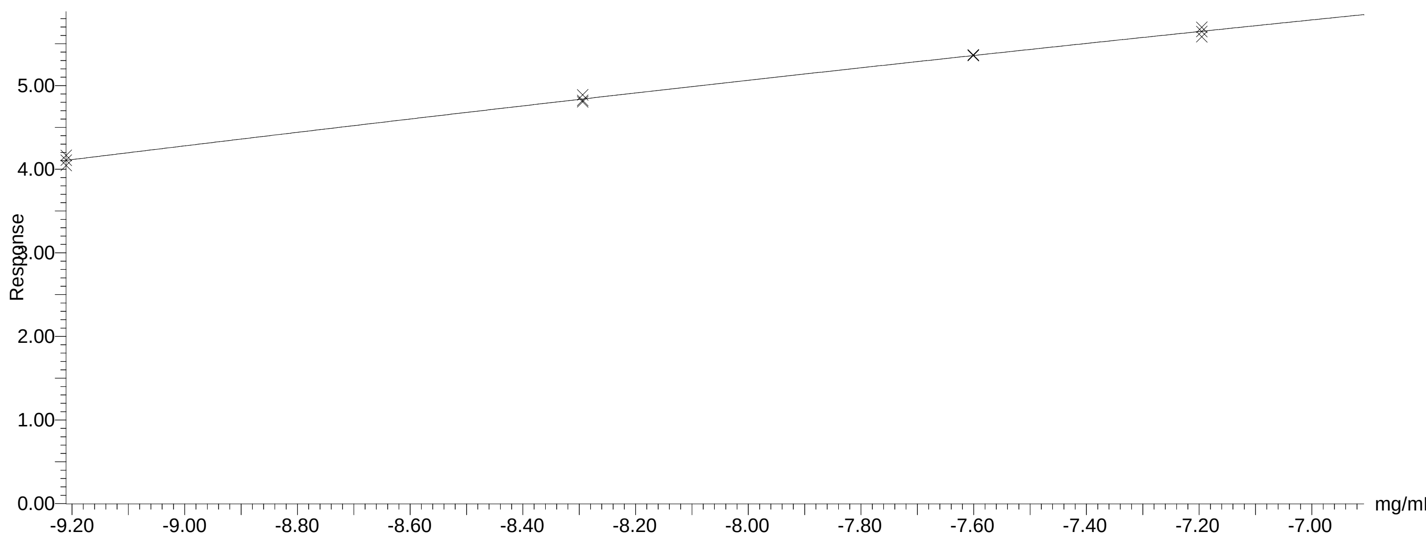
Quantify Calibration Report **MassLynx 4.1** Page 1 of 1

Dataset: Untitled

Last Altered: Tuesday, January 08, 2008 16:29:22 Eastern Standard Time
Printed: At Tuesday, January 08, 2008 16:29:57 Eastern Standard Time
By WILNT\acgenuser (acgenuser)

Method: \\Lcms02\LCMS02\189207.PRO\MethDB\I2-189207a.mdb 08 Jan 2008 14:34:39
Calibration: 08 Jan 2008 16:29:22

Compound name: HFPO dimer acid ammonium salt
Coefficient of Determination: R^2 = 0.996687
Calibration curve: -0.0312312 * x^2 + 0.253593 * x + 9.0911
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: Null, Axis trans: Ln



Dataset: Z:\189207.PRO\I3-189207d_r1.qld

Last Altered: Monday, April 14, 2008 13:38:09 Eastern Daylight Time
Printed: At Monday, April 14, 2008 13:51:41 Eastern Daylight Time
By WILNT\hiller (Josh Hiller)

Method: Z:\189207.pro\MethDB\189207.mdb 17 Dec 2007 21:49:05
Calibration: 14 Apr 2008 13:38:09

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
1	I3-0325	47-7	STD 500ng		0.59	12492.672		12492.67200	bb	1.000	0.0005	
2	I3-0326	47-7	STD 500ng		0.61	11943.259		11943.25900	bb	1.000	0.0005	
3	I3-0327	47-7	STD 500ng		0.61	12169.127		12169.12700	bb	1.000	0.0005	
4	I3-0328	47-7	STD 500ng		0.61	12341.121		12341.12100	bb	1.000	0.0005	
5	I3-0329	47-7	STD 500ng		0.61	12465.429		12465.42900	bb	1.000	0.0005	
6	I3-0330	47-7	STD 500ng		0.61	12282.093		12282.09300	bb	1.000	0.0005	
7	I3-0331	47-7	STD 500ng		0.61	12625.358		12625.35800	bb	1.000	0.0005	
8	I3-0332	47-7	STD 500ng		0.61	12725.863		12725.86300	bb	1.000	0.0005	
9	I3-0333	47-7	STD 500ng		0.61	12047.822		12047.82200	bb	1.000	0.0005	
10	I3-0334		Diluent		0.04	19.828		19.82800	bb	1.000	0.0000	
11	I3-0335	47-1	STD 100ng	0.00	0.61	3577.054		3577.05400	bb	1.000	0.0001	1.247
12	I3-0336	47-4	STD 250ng	0.00	0.61	7805.657		7805.65700	bb	1.000	0.0003	4.289
13	I3-0337	47-7	STD 500ng	0.00	0.61	12587.336		12587.33600	bb	1.000	0.0005	-0.569
14	I3-0338	47-10	STD 750ng	0.00	0.61	16455.465		16455.46500	bb	1.000	0.0007	-1.990
15	I3-0339	47-13	STD 1000ng	0.00	0.61	19751.744		19751.74400	bb	1.000	0.0010	-2.630
16	I3-0340		Diluent		0.04	44.871		44.87100	bb	1.000	0.0000	
17	I3-0341	36-1	blank		0.61	3119.999		3119.99900	bb	2.000	0.0002	
18	I3-0342	36-2	QC .001mg	0.00	0.61	15665.404		15665.40400	bb	2.000	0.0014	36.581
19	I3-0343	36-5	QC .05 mg	0.05	0.61	15149.693		15149.69300	bb	100.0...	0.0650	29.972
20	I3-0344	37-1	QC .5 mg	0.50	0.61	14583.725		14583.72500	bb	1000....	0.6145	22.899
21	I3-0345	37-4	QC 50 mg	50.00	0.61	12769.289		12769.28900	bb	1000...	50.7381	1.476
22	I3-0346		Diluent		0.61	17.243		17.24300	bb	1.000	0.0000	
23	I3-0347	39-1	Low, Stab, 0.01mg/mL		0.56	11.287		11.28700	bb	20.000	0.0000	-99.869
24	I3-0348	39-2a	Low, Stab, 0.01mg/mL		0.61	12442.148		12442.14800	bb	20.000	0.0098	-2.187
25	I3-0349	39-3a	Low, Stab, 0.01mg/mL		0.61	13468.224		13468.22400	bb	20.000	0.0110	9.506
26	I3-0350	39-4	Low, Stab, 0.01mg/mL		0.61	18336.240		18336.24000	bb	20.000	0.0173	73.400
27	I3-0351		Diluent		0.61	16.895		16.89500	bb	1.000	0.0000	
28	I3-0352		Diluent		0.61	17.180		17.18000	bb	1.000	0.0000	
29	I3-0353	39-5	High, Stab, 3 mg/mL		0.61	12263.065		12263.06500	bb	6000....	2.8750	-4.168
30	I3-0354	39-6	High, Stab, 3 mg/mL		0.61	12360.235		12360.23500	bb	6000....	2.9071	-3.095
31	I3-0355	39-7	High, Stab, 3 mg/mL		0.61	12456.538		12456.53800	bb	6000....	2.9392	-2.028
32	I3-0356	39-8	High, Stab, 3 mg/mL		0.61	12341.143		12341.14300	bb	6000....	2.9008	-3.307
33	I3-0357		Diluent		0.05	13.254		13.25400	bb	1.000	0.0000	

Dataset: Z:\189207.PRO\I3-189207d_r1.qld

Last Altered: Monday, April 14, 2008 13:38:09 Eastern Daylight Time
Printed: At Monday, April 14, 2008 13:51:41 Eastern Daylight Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
34	I3-0358	36-3	QC .001mg	0.00	0.61	14732.837		14732.83700	bb	2.000	0.0012	24.744
35	I3-0359	36-6	QC .05 mg	0.05	0.61	16569.266		16569.26600	bb	100.0...	0.0743	48.550
36	I3-0360	37-2	QC .5 mg	0.50	0.62	14525.371		14525.37100	bb	1000....	0.6109	22.181
37	I3-0361	37-5	QC 50 mg	50.00	0.61	13193.270		13193.27000	bb	1000...	53.1570	6.314
38	I3-0362		Diluent		0.62	17.909		17.90900	bb	1.000	0.0000	
39	I3-0363	47-2	STD 100ng	0.00	0.61	3553.047		3553.04700	bb	1.000	0.0001	0.471
40	I3-0364	47-5	STD 250ng	0.00	0.61	7265.604		7265.60400	bb	1.000	0.0002	-4.875
41	I3-0365	47-8	STD 500ng	0.00	0.61	12324.365		12324.36500	bb	1.000	0.0005	-3.492
42	I3-0366	47-11	STD 750ng	0.00	0.61	18234.566		18234.56600	bb	1.000	0.0009	14.611
43	I3-0367	47-14	STD 1000ng	0.00	0.61	20073.014		20073.01400	bb	1.000	0.0010	-0.117
44	I3-0368		Diluent		0.05	36.525		36.52500	bb	1.000	0.0000	
45	I3-0369	42-3	Grp 2, .01mg/mL		0.61	13033.570		13033.57000	bb	20.000	0.0104	4.480
46	I3-0370	42-4	Grp 2, .01mg/mL		0.61	13710.542		13710.54200	bb	20.000	0.0112	12.354
47	I3-0371	43-1	Grp 3, 0.3 mg/mL		0.61	14154.519		14154.51900	bb	600.0...	0.3530	17.661
48	I3-0372	43-2	Grp 3, 0.3 mg/mL		0.61	13489.919		13489.91900	bb	600.0...	0.3293	9.759
49	I3-0373	43-3	Grp 4, 3.0 mg/mL		0.61	12460.103		12460.10300	bb	6000....	2.9404	-1.988
50	I3-0374	43-4	Grp 4, 3.0 mg/mL		0.61	12660.408		12660.40800	bb	6000....	3.0075	0.250
51	I3-0375		Diluent		0.61	15.484		15.48400	bb	1.000	0.0000	
52	I3-0376	36-4	QC .001mg	0.00	0.61	15534.162		15534.16200	bb	2.000	0.0013	34.884
53	I3-0377	36-7	QC .05 mg	0.05	0.61	15937.784		15937.78400	bb	100.0...	0.0701	40.136
54	I3-0378	37-3	QC .5 mg	0.50	0.61	13900.369		13900.36900	bb	1000....	0.5730	14.609
55	I3-0379	37-6	QC 50 mg	50.00	0.61	13180.649		13180.64900	bb	1000...	53.0842	6.168
56	I3-0380		Diluent		0.61	17.252		17.25200	bb	1.000	0.0000	
57	I3-0381	47-3	STD 100ng	0.00	0.61	3493.359		3493.35900	bb	1.000	0.0001	-1.451
58	I3-0382	47-6	STD 250ng	0.00	0.61	7585.784		7585.78400	bb	1.000	0.0003	0.522
59	I3-0383	47-9	STD 500ng	0.00	0.61	12721.201		12721.20100	bb	1.000	0.0005	0.934
60	I3-0384	47-12	STD 750ng	0.00	0.61	16228.391		16228.39100	bb	1.000	0.0007	-4.015
61	I3-0385	47-15	STD 1000ng	0.00	0.62	19921.584		19921.58400	bb	1.000	0.0010	-1.305
62	I3-0386		Diluent		0.05	36.437		36.43700	bb	1.000	0.0000	
63	I3-0387		Diluent		0.61	18.854		18.85400	bb	1.000	0.0000	
64	I3-0388		Diluent		0.61	15.391		15.39100	bb	1.000	0.0000	
65	I3-0389		Diluent		0.61	17.074		17.07400	bb	1.000	0.0000	
66	I3-0390		Diluent		0.61	15.578		15.57800	bb	1.000	0.0000	
67	I3-0391		Diluent		0.61	16.251		16.25100	bb	1.000	0.0000	
68	I3-0392	42-1	Grp 1, blank		0.61	21.468		21.46800	bb	1.000	0.0000	
69	I3-0393	42-2	Grp 1, blank		0.61	25.433		25.43300	bb	1.000	0.0000	

Quantify Compound Summary Report MassLynx 4.1

Dataset: Z:\189207.PRO\I3-189207d_r1.qld

Last Altered: Monday, April 14, 2008 13:38:09 Eastern Daylight Time
Printed: At Monday, April 14, 2008 13:51:41 Eastern Daylight Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
70	I3-0394		Diluent		0.62	14.843		14.84300	bb	1.000	0.0000	
71	I3-0395		Diluent		0.62	15.278		15.27800	bb	1.000	0.0000	
72	I3-0396		Diluent		0.61	17.829		17.82900	bb	1.000	0.0000	
73	I3-0397	177-2	QC .001 mg		0.61	12671.216		12671.21600	bb	2.000	0.0010	0.372
74	I3-0398	177-3	QC .001 mg		0.61	13219.157		13219.15700	bb	2.000	0.0011	6.613
75	I3-0399	177-4	QC .001 mg		0.61	12697.865		12697.86500	bb	2.000	0.0010	0.671
76	I3-0400	177-5	QC .05 mg		0.61	12782.253		12782.25300	bb	100.0...	0.0508	1.623
77	I3-0401	177-6	QC .05 mg		0.61	12817.794		12817.79400	bb	100.0...	0.0510	2.025
78	I3-0402	177-7	QC .05 mg		0.61	12369.916		12369.91600	bb	100.0...	0.0485	-2.988
79	I3-0403	178-1	QC .5 mg		0.61	13494.399		13494.39900	bb	1000....	0.5491	9.812
80	I3-0404	178-2	QC .5 mg		0.61	12863.526		12863.52600	bb	1000....	0.5127	2.543
81	I3-0405	178-3	QC .5 mg		0.61	13416.332		13416.33200	bb	1000....	0.5445	8.900
82	I3-0406	178-4	QC 50 mg		0.61	13289.744		13289.74400	bb	1000...	53.7145	7.429
83	I3-0407	178-5	QC 50 mg		0.61	13027.686		13027.68600	bb	1000...	52.2063	4.413
84	I3-0408	178-6	QC 50 mg		0.61	12801.501		12801.50100	bb	1000...	50.9201	1.840
85	I3-0409		Diluent		0.61	20.342		20.34200	bb	1.000	0.0000	
86	I3-0410		Diluent		0.61	21.491		21.49100	bb	1.000	0.0000	
87	I3-0411		Diluent		0.61	21.295		21.29500	bb	1.000	0.0000	
88	I3-0412	39-1	Low, Stab, 0.01mg/mL		0.61	19169.131		19169.13100	bb	20.000	0.0186	85.799
89	I3-0413	39-2a	Low, Stab, 0.01mg/mL		0.61	12748.167		12748.16700	bb	20.000	0.0101	1.238
90	I3-0414	39-3a	Low, Stab, 0.01mg/mL		0.61	13633.857		13633.85700	bb	20.000	0.0111	11.449
91	I3-0415	39-4	Low, Stab, 0.01mg/mL		0.61	18986.916		18986.91600	bb	20.000	0.0183	83.048
92	I3-0416		Diluent		0.61	7288.208		7288.20800	bb	1.000	0.0002	
93	I3-0417	39-1a	Low, Stab, 0.01mg/mL		0.61	11909.852		11909.85200	bb	20.000	0.0092	-8.021
94	I3-0418	39-4a	Low, Stab, 0.01mg/mL		0.62	11816.611		11816.61100	bb	20.000	0.0091	-9.027
95	I3-0419		Diluent		0.61	23.110		23.11000	bb	1.000	0.0000	
96	I3-0420	50-1	blank		0.62	23.508		23.50800	bb	2.000	0.0000	
97	I3-0421	50-2	QC .001mg	0.00	0.61	12437.376		12437.37600	bb	2.000	0.0010	-2.240
98	I3-0422	50-3	QC .001mg		0.61	13193.417		13193.41700	bb	2.000	0.0011	6.316
99	I3-0423	50-4	QC .001mg		0.61	12665.197		12665.19700	bb	2.000	0.0010	0.304
100	I3-0424	50-5	QC .05 mg	0.05	0.62	12793.309		12793.30900	bb	100.0...	0.0509	1.748
101	I3-0425	50-6	QC .05 mg	0.05	0.62	12788.629		12788.62900	bb	100.0...	0.0508	1.695
102	I3-0426	50-7	QC .05 mg	0.05	0.61	12702.056		12702.05600	bb	100.0...	0.0504	0.718
103	I3-0427	51-1	QC .5 mg	0.50	0.61	13737.457		13737.45700	bb	1000....	0.5634	12.673
104	I3-0428	51-2	QC .5 mg	0.50	0.62	12721.494		12721.49400	bb	1000....	0.5047	0.937
105	I3-0429	51-3	QC .5 mg	0.50	0.61	12389.452		12389.45200	bb	1000....	0.4861	-2.772

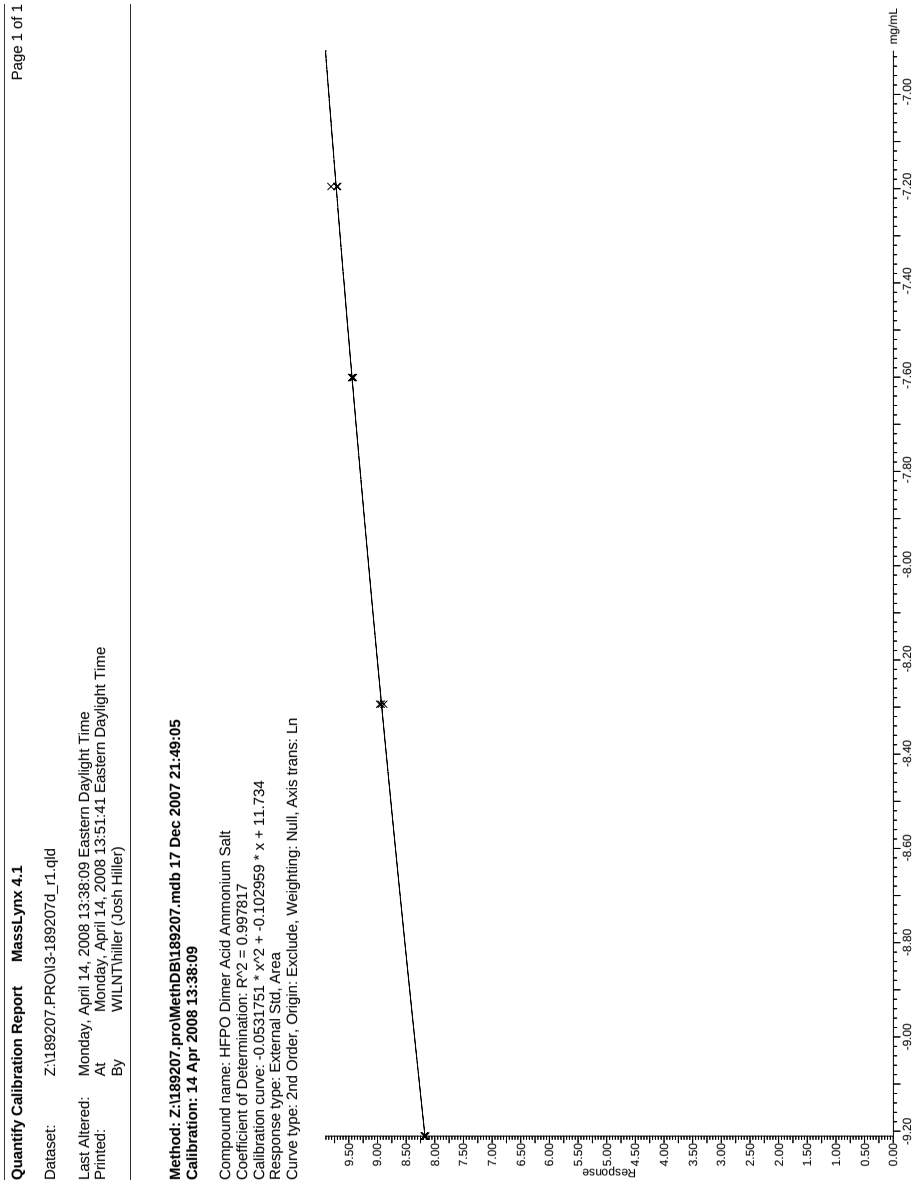
Quantify Compound Summary Report **MassLynx 4.1**

Dataset: Z:\189207.PRO\I3-189207d_r1.qld

Last Altered: Monday, April 14, 2008 13:38:09 Eastern Daylight Time
Printed: At Monday, April 14, 2008 13:51:41 Eastern Daylight Time
By WILNT\hiller (Josh Hiller)

Compound name: HFPO Dimer Acid Ammonium Salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	IS Area	Response	Flags	Mult.	mg/mL	% RE
106	I3-0430	51-4	QC 50 mg	50.00	0.61	12284.569		12284.56900	bb	1000...	48.0346	-3.931
107	I3-0431	51-5	QC 50 mg	50.00	0.61	12392.128		12392.12800	bb	1000...	48.6288	-2.742
108	I3-0432	51-6	QC 50 mg	50.00	0.61	12956.257		12956.25700	bb	1000...	51.7985	3.597
109	I3-0433		Diluent		0.61	20.950		20.95000	bb	1.000	0.0000	
110	I3-0434	39-1a	Low, Stab, 0.01mg/mL		0.61	12520.454		12520.45400	bb	20.000	0.0099	-1.316
111	I3-0435	39-2a	Low, Stab, 0.01mg/mL		0.61	12403.895		12403.89500	bb	20.000	0.0097	-2.612
112	I3-0436	39-3a	Low, Stab, 0.01mg/mL		0.61	12724.229		12724.22900	bb	20.000	0.0101	0.968
113	I3-0437	39-4a	Low, Stab, 0.01mg/mL		0.61	13261.877		13261.87700	bb	20.000	0.0107	7.106


Quantify Calibration Report MassLynx 4.1
Page 1 of 1
Dataset: Z:\189207.PROI\3-189207d_r1.qld
Last Altered: Monday, April 14, 2008 13:38:09 Eastern Daylight Time
Printed: At Monday, April 14, 2008 13:51:41 Eastern Daylight Time
By WILNT\hiller (Josh Hiller)
Method: Z:\189207.proIMethDB\189207.mdb 17 Dec 2007 21:49:05
Calibration: 14 Apr 2008 13:38:09
Compound name: HFPO Dimer Acid Ammonium Salt
Coefficient of Determination: R^2 = 0.997817
Calibration curve: -0.0531751 * x^2 + -0.102959 * x + 11.734
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: Null, Axis trans: Ln
Response
mg/mL

Quantify Compound Summary Report   MassLynx 4.1

Dataset: C:\MassLynx\189205.PRO\189205f.qld

Last Altered: Wednesday, January 02, 2008 14:59:24 Eastern Standard Time
Printed: At Wednesday, January 02, 2008 15:15:20 Eastern Standard Time
By WILNT\acgenuser (acgenuser)

Method: C:\MassLynx\189205.PRO\MethDB\I2-189205a.mdb 16 Nov 2007 17:22:48
Calibration: 02 Jan 2008 14:59:24

Compound name: HFPO dimer acid ammonium salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
1	I2-0194	200-10	System Suit		0.57	623.6	623.6	bb	1.00	0.000004316	
2	I2-0195	200-10	System Suit						1.00		
3	I2-0196	200-10	System Suit		0.57	531.5	531.5	bb	1.00	0.000003819	
4	I2-0197	200-10	System Suit						1.00		
5	I2-0198		Diluent		0.62	568.7	568.7	bb	1.00	0.000004022	
6	I2-0199	200-1	STD 100ng		0.62	20260	20260	bb	1.00	0.00009989	-0.114
7	I2-0200	200-4	STD 250ng		0.62	44480	44480	bb	1.00	0.0002485	-0.596
8	I2-0201	200-7	STD 500ng		0.62	77330	77330	bb	1.00	0.0005125	2.51
9	I2-0202	200-10	STD 750ng						1.00		
10	I2-0203	200-13	STD 1000ng		0.62	121600	121600	bb	1.00	0.0009979	-0.209
11	I2-0204		Diluent		0.62	122700	122700	bb	1.00	0.001011	
12	I2-0205	189207-50-1	QC .001mg	0.00100	0.62	326.2	326.2	bb	2.00	0.000005299	-99.5
13	I2-0206	189207-50-4	QC .05 mg	0.0500	0.62	75280	75280	bb	100	0.04939	-1.23
14	I2-0207	189207-51-1	QC .5 mg	0.500	0.63	76180	76180	bb	1000	0.5021	0.415
15	I2-0208	189207-51-4	QC 50 mg	50.0	0.62	75850	75850	bb	100000	49.90	-0.204
16	I2-0209		Diluent		0.62	75970	75970	bb	1.00	0.0005001	
17	I2-0210	201-1	Group 1		0.63	175.9	175.9	bb	1.00	0.000001695	
18	I2-0211	201-2	Group 1		0.62	155.6	155.6	bb	1.00	0.000001555	
19	I2-0212	202-1	Group 2M		0.62	72590	72590	bb	60.0	0.02818	-6.06

Quantify Compound Summary Report **MassLynx 4.1** Page 2 of 3

Dataset: C:\MassLynx\189205.PRO\189205f.qld

Last Altered: Wednesday, January 02, 2008 14:59:24 Eastern Standard Time
Printed: At Wednesday, January 02, 2008 15:15:20 Eastern Standard Time
By WILNT\acgenuser (acgenuser)

Compound name: HFPO dimer acid ammonium salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
20	I2-0213	202-2	Group 2M		0.62	72580	72580	bb	60.0	0.02818	-6.07
21	I2-0214	202-3	Group 2F/3M		0.62	75380	75380	bb	600	0.2968	-1.05
22	I2-0215	202-4	Group 2F/3M		0.62	74910	74910	bb	600	0.2943	-1.89
23	I2-0216	202-5	Group 4M		0.62	73090	73090	bb	6000	2.845	-5.16
24	I2-0217	202-6	Group 4M		0.62	76680	76680	bb	6000	3.039	1.31
25	I2-0218	202-7	Group 3F		0.62	76240	76240	bb	6000	3.015	0.511
26	I2-0219	202-8	Group 3F		0.62	76460	76460	bb	6000	3.028	0.926
27	I2-0220	202-9	Group 4F		0.62	75820	75820	bb	60000	29.93	-0.245
28	I2-0221	202-10	Group 4F		0.62	75650	75650	bb	60000	29.83	-0.566
29	I2-0222		Diluent		0.62	610.5	610.5	bb	60000	0.2548	-99.2
30	I2-0223	189207-50-2	QC .001mg	0.00100	0.62	76270	76270	bb	2.00	0.001006	0.567
31	I2-0224	189207-50-5	QC .05 mg	0.0500	0.62	76530	76530	bb	100	0.05052	1.04
32	I2-0225	189207-51-2	QC .5 mg	0.500	0.62	76770	76770	bb	1000	0.5075	1.49
33	I2-0226	189207-51-5	QC 50 mg	50.0	0.62	73980	73980	bb	100000	48.22	-3.57
34	I2-0227		Diluent		0.62	620.5	620.5	bb	1.00	0.000004300	
35	I2-0228	200-2	STD 100ng	0.000100	0.62	20500	20500	bb	1.00	0.0001012	1.20
36	I2-0229	200-5	STD 250ng	0.000250	0.62	45040	45040	bb	1.00	0.0002524	0.942
37	I2-0230	200-8	STD 500ng	0.000500	0.62	75930	75930	bb	1.00	0.0004998	-0.0434
38	I2-0231	200-11	STD 750ng	0.000750	0.62	101400	101400	bb	1.00	0.0007559	0.780
39	I2-0232	200-14	STD 1000ng	0.00100	0.62	122500	122500	bb	1.00	0.001009	0.904
40	I2-0233		Diluent		0.62	647.5	647.5	bb	60000	0.2666	
41	I2-0234	189207-50-3	QC .001mg	0.00100	0.62	74530	74530	bb	2.00	0.0009742	-2.58

Quantify Compound Summary Report **MassLynx 4.1**

Dataset: C:\MassLynx\189205.PRO\189205f.qld

Last Altered: Wednesday, January 02, 2008 14:59:24 Eastern Standard Time
Printed: At Wednesday, January 02, 2008 15:15:20 Eastern Standard Time
By WILNT\acgenuser (acgenuser)

Compound name: HFPO dimer acid ammonium salt

	Run #	Ref #	Sample Text	Std. Conc.	RT	Area	Response	Flags	Mult.	mg/mL	% RE
42	I2-0235	189207-50-6	QC .05 mg	0.0500	0.62	74690	74690	bb	100	0.04885	-2.30
43	I2-0236	189207-51-3	QC .5 mg	0.500	0.62	74870	74870	bb	1000	0.4901	-1.97
44	I2-0237	189207-51-6	QC 50 mg	50.0	0.62	73880	73880	bb	100000	48.13	-3.75
45	I2-0238		Diluent		0.62	608.6	608.6	bb	1.00	0.000004236	
46	I2-0239	200-3	STD 100ng	0.000100	0.62	20090	20090	bb	1.00	0.00009899	-1.01
47	I2-0240	200-6	STD 250ng	0.000250	0.62	44190	44190	bb	1.00	0.0002465	-1.40
48	I2-0241	200-9	STD 500ng	0.000500	0.62	75580	75580	bb	1.00	0.0004966	-0.685
49	I2-0242	200-12	STD 750ng	0.000750	0.62	102200	102200	bb	1.00	0.0007651	2.01
50	I2-0243	200-15	STD 1000ng	0.00100	0.62	119800	119800	bb	1.00	0.0009741	-2.59
51	I2-0244		Diluent		0.62	649.2	649.2	bb	1.00	0.000004453	

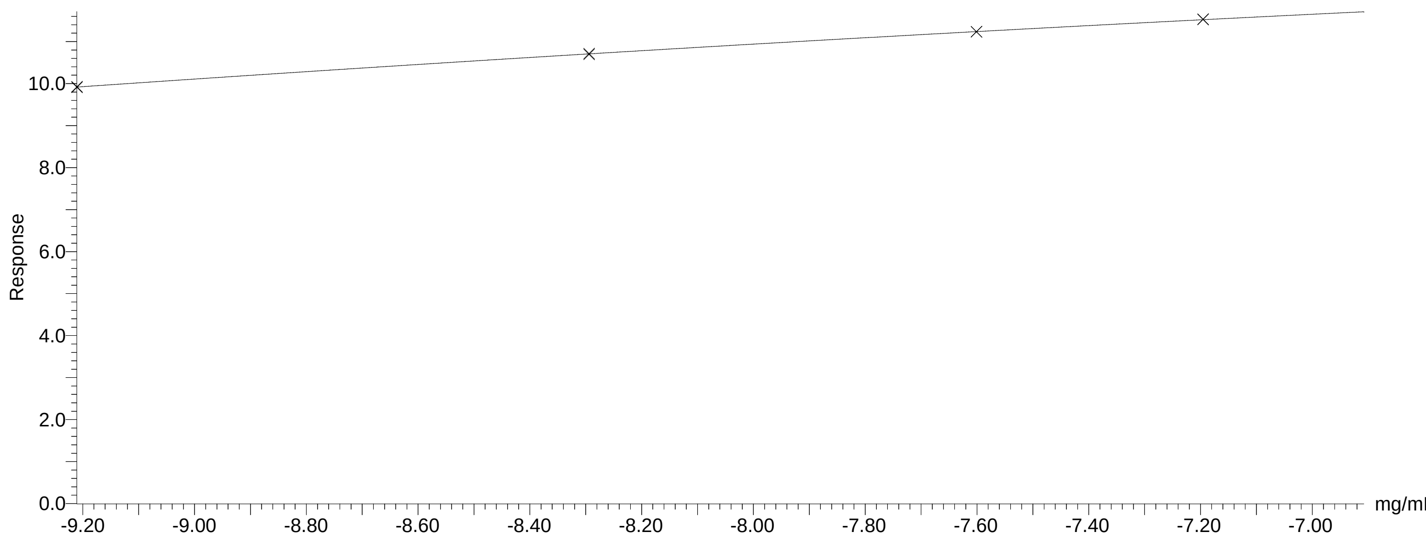
Quantify Calibration Report **MassLynx 4.1**

Dataset: C:\MassLynx\189205.PRO\189205f.qld

Last Altered: Wednesday, January 02, 2008 14:59:24 Eastern Standard Time
Printed: At Wednesday, January 02, 2008 15:15:20 Eastern Standard Time
By WILNT\acgenuser (acgenuser)

Method: C:\MassLynx\189205.PRO\MethDB\I2-189205a.mdb 16 Nov 2007 17:22:48
Calibration: 02 Jan 2008 14:59:24

Compound name: HFPO dimer acid ammonium salt
Coefficient of Determination: R^2 = 0.999775
Calibration curve: -0.0604991 * x^2 + -0.196635 * x + 13.2386
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: Null, Axis trans: Ln



APPENDIX D

Pretest Clinical Observations

PRETEST CLINICAL OBSERVATIONS

PROJECT NO.:WIL-189207P — A 28-DAY ORAL STUDY OF H-28397 IN MICE — PAGE 1
SPONSOR:E.I. DUPONT — SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS
SPONSOR NO.:DUPONT-24459

	----- M A L E -----
TABLE RANGE:	12-07-07 TO 12-18-07
GROUP:	1
NORMAL	
-NO SIGNIFICANT CLINICAL OBSERVATIONS	140/68
BODY/INTEGUMENT	
-HAIR LOSS DORSAL RUMP	1/ 1
EYES/EARS/NOSE	
-ABNORMAL PUPIL POSITION LEFT EYE	4/ 4
-ABNORMAL PUPIL POSITION RIGHT EYE	4/ 3
BODY/INTEG II	
-SCABBING DORSAL RUMP	3/ 1
-SCABBING UROGENITAL AREA	1/ 1
SPECIAL	
-SWOLLEN UROGENITAL AREA	1/ 1
SPECIAL II	
-PROLAPSED PENIS	1/ 1
-WATER BOTTLE ADDED - PHYSICAL CONDITION	1/ 1

1- PRETEST

PRETEST CLINICAL OBSERVATIONS

PROJECT NO.:WIL-189207P — A 28-DAY ORAL STUDY OF H-28397 IN MICE —

SPONSOR:E.I. DUPONT — SUMMARY OF CLINICAL FINDINGS: TOTAL OCCURRENCE/NO. OF ANIMALS

SPONSOR NO.:DUPONT-24459

----- F E M A L E -----

TABLE RANGE: 12-07-07 TO 12-18-07	
GROUP:	1
NORMAL	
-NO SIGNIFICANT CLINICAL OBSERVATIONS	144/69
EYES/EARS/NOSE	
-ABNORMAL PUPIL POSITION RIGHT EYE	4/ 3
BODY/INTEG II	
-SCABBING FORELIMB(S)	1/ 1
-SCABBING LEFT EAR	1/ 1

1- PRETEST

PCSUv4.07
05/21/2008

APPENDIX E

Unscheduled Clinical Observations

PROJECT NO.:WIL-189207V
SPONSOR:E.I. DUPONT
SPONSOR NO.:DUPONT-24459

UNSCHEDULED CLINICAL OBSERVATIONS
A 28-DAY ORAL STUDY OF H-28397 IN MICE
INDIVIDUAL CLINICAL OBSERVATIONS

STUDY DAYS: 0 THROUGH 58

ANIMAL	SEX	GROUP	CATEGORY	STUDY DAY	TIME	GRADE	OBSERVATIONS
91015	F	0 MG/KG/DAY	SPECIAL II	21	12:11	P	WATER BOTTLE ADDED-POOR BODY CONDITION
91007	F	30 MG/KG/DAY	EYES/EARS/NOSE	34	9:33	P	COMPLETE CLOSURE RIGHT EYE
91007	F	30 MG/KG/DAY	SPECIAL	34	9:05	P	SWOLLEN FACIAL AREA
				34	9:05	P	SWOLLEN DORSAL NECK
91007	F	30 MG/KG/DAY	SPECIAL II	34	9:16	P	MASS, FACIAL AREA, 6MM X 4MM X 3MM, SMOOTH, FIRM, IMMOVABLE, DISCOLORED
				34	9:19	P	MASS, FACIAL AREA, 9MM X 6MM X 4MM, SMOOTH, FIRM, IMMOVABLE, DISCOLORED, PURULENT DISCHARGE
				34	9:28	P	MASS, DORSAL NECK, 12MM X 10MM X 6MM, SMOOTH, SOFT, MOVABLE, NORMAL SURFACE
				34	9:30	P	MASS, DORSAL BACK, 10MM X 6MM X 4MM, SMOOTH, SOFT, MOVABLE, NORMAL SURFACE

GRADE CODE: 1 - SLIGHT 2 - MODERATE 3 - SEVERE P - PRESENT

PCRDv4.11
04/21/2008

APPENDIX F

Animal Room Environmental Conditions

A 28-DAY ORAL STUDY OF H-28397 IN MICE

PROJECT NO.:WIL- 189207 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY

SPONSOR: E.I. DUPONT

STUDY SPECIFICATIONS: 189207 DATE IN: 12/04/07 TIME IN: 7:00
DATE OUT: 02/14/08 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 38 LOW TEMPERATURE ºF: 66.0 HIGH TEMPERATURE ºF: 76.0 LOW HUMIDITY: 30.0
SPECIES: MOUSE LOW TEMPERATURE ºC: 18.9 HIGH TEMPERATURE ºC: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (ºF)	MEAN (ºC)	MEAN (%RH)
04-Dec-07	70.1	21.2	41.4
05-Dec-07	70.5	21.4	41.8
06-Dec-07	70.7	21.5	40.5
07-Dec-07	70.7	21.5	41.7
08-Dec-07	70.6	21.4	41.5
09-Dec-07	70.5	21.4	41.7
10-Dec-07	70.5	21.4	42.2
11-Dec-07	70.6	21.4	45.3
12-Dec-07	70.5	21.4	43.0
13-Dec-07	70.6	21.4	42.7
14-Dec-07	70.4	21.3	42.8
15-Dec-07	70.6	21.5	42.0
16-Dec-07	70.3	21.3	41.8
17-Dec-07	70.5	21.4	41.4
18-Dec-07	70.5	21.4	41.8
19-Dec-07	70.5	21.4	43.1
20-Dec-07	70.5	21.4	42.3
21-Dec-07	70.5	21.4	43.0
22-Dec-07	70.4	21.3	42.5
23-Dec-07	70.6	21.4	42.5
24-Dec-07	70.5	21.4	41.0
25-Dec-07	70.6	21.4	41.5
26-Dec-07	70.5	21.4	41.9

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
\- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

REPORT 4
VERSION 1.09
4/1/2008 13:32

A 28-DAY ORAL STUDY OF H-28397 IN MICE

PROJECT NO.:WIL- 189207 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY
SPONSOR: E.I. DUPONT

STUDY SPECIFICATIONS: 189207 DATE IN: 12/04/07 TIME IN: 7:00
DATE OUT: 02/14/08 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 38 LOW TEMPERATURE ºF: 66.0 HIGH TEMPERATURE ºF: 76.0 LOW HUMIDITY: 30.0
SPECIES: MOUSE LOW TEMPERATURE ºC: 18.9 HIGH TEMPERATURE ºC: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (ºF)	MEAN (ºC)	MEAN (%RH)
27-Dec-07	70.4	21.4	42.0
28-Dec-07	70.6	21.4	42.2
29-Dec-07	70.5	21.4	42.0
30-Dec-07	70.5	21.4	41.7
31-Dec-07	70.7	21.5	41.9
01-Jan-08	70.6	21.4	41.2
02-Jan-08	70.3	21.3	41.4
03-Jan-08	70.4	21.3	41.1
04-Jan-08	70.5	21.4	42.3
05-Jan-08	70.6	21.5	42.3
06-Jan-08	70.5	21.4	43.5
07-Jan-08	70.5	21.4	50.0
08-Jan-08	70.4	21.3	47.2
09-Jan-08	70.5	21.4	43.8
10-Jan-08	70.6	21.5	42.1
11-Jan-08	70.6	21.4	42.9
12-Jan-08	70.5	21.4	42.0
13-Jan-08	70.5	21.4	41.9
14-Jan-08	70.4	21.3	40.9
15-Jan-08	70.6	21.5	39.9
16-Jan-08	70.5	21.4	40.0
17-Jan-08	70.5	21.4	40.5
18-Jan-08	70.4	21.3	40.7

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
\- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

A 28-DAY ORAL STUDY OF H-28397 IN MICE

PROJECT NO.:WIL- 189207 TEMPERATURE/HUMIDITY - DAILY SUMMARY REPORT BY STUDY

SPONSOR: E.I. DUPONT

STUDY SPECIFICATIONS: 189207 DATE IN: 12/04/07 TIME IN: 7:00
DATE OUT: 02/14/08 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 38 LOW TEMPERATURE ºF: 66.0 HIGH TEMPERATURE ºF: 76.0 LOW HUMIDITY: 30.0
SPECIES: MOUSE LOW TEMPERATURE ºC: 18.9 HIGH TEMPERATURE ºC: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (ºF)	MEAN (ºC)	MEAN (%RH)
19-Jan-08	70.7	21.5	39.6
20-Jan-08	70.4	21.3	39.6
21-Jan-08	70.5	21.4	39.8
22-Jan-08	70.5	21.4	41.1
23-Jan-08	70.4	21.3	40.0
24-Jan-08	70.6	21.5	40.2
25-Jan-08	70.6	21.4	38.5
26-Jan-08	70.6	21.4	37.7
27-Jan-08	70.6	21.5	37.6
28-Jan-08	70.5	21.4	38.0
29-Jan-08	70.5	21.4	39.4
30-Jan-08	70.5	21.4	37.4
31-Jan-08	70.4	21.4	37.9
01-Feb-08	70.6	21.4	38.5
02-Feb-08	70.5	21.4	38.4
03-Feb-08	70.5	21.4	38.5
04-Feb-08	70.6	21.5	39.3
05-Feb-08	70.5	21.4	42.2
06-Feb-08	70.5	21.4	39.0
07-Feb-08	70.5	21.4	38.6
08-Feb-08	70.5	21.4	38.1
09-Feb-08	70.5	21.4	38.4
10-Feb-08	70.4	21.4	35.5

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
\- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

REPORT 4
VERSION 1.09
4/1/2008 13:32

STUDY SPECIFICATIONS: 189207 DATE IN: 12/04/07 TIME IN: 7:00
DATE OUT: 02/14/08 TIME OUT: 16:00

ROOM SPECIFICATIONS: B ROOM 38 LOW TEMPERATURE ºF: 66.0 HIGH TEMPERATURE ºF: 76.0 LOW HUMIDITY: 30.0
SPECIES: MOUSE LOW TEMPERATURE ºC: 18.9 HIGH TEMPERATURE ºC: 24.4 HIGH HUMIDITY: 70.0

	TEMPERATURE		HUMIDITY
DATE	MEAN (ºF)	MEAN (ºC)	MEAN (%RH)
11-Feb-08	70.4	21.3	37.9
12-Feb-08	70.5	21.4	38.0
13-Feb-08	70.4	21.4	38.4
14-Feb-08	70.5	21.4	38.7

GRAND STATS	MEAN	MIN	MAX
TEMPERATURE ºF	70.5	70.1	70.7
TEMPERATURE ºC	21.4	21.2	21.5
HUMIDITY (%RH)	41.0	35.5	50.0
N DAYS	73		

NOTE: + = VALUE WAS GREATER THAN HIGH RANGE
\- = VALUE WAS LESS THAN LOW RANGE
NOTE: MEANS REPRESENT THE MEAN OF THE DAILY VALUES

907
WIL-189207
A 28-Day Oral (Gavage) Toxicity Study
Of H-28397 In Mice With A 28-Day Recovery
DuPont-24459

A 28-DAY ORAL STUDY OF H-28397 IN MICE

PROJECT NO.:WIL- 189207 TEMPERATURE/HUMIDITY - END OF STUDY SUMMARY REPORT 13:31 01-Apr-08 PAGE 1

SPONSOR: E.I. DUPONT

ROOM SPECIFICATIONS:	B ROOM 38		
SPECIES:	MOUSE		
LOW TEMPERATURE:	66.0	DATE IN:	12/04/07
HIGH TEMPERATURE:	76.0	TIME IN:	7:00
LOW HUMIDITY:	30.0	DATE OUT:	02/14/08
HIGH HUMIDITY:	70.0	TIME OUT:	16:00

ROOM B ROOM 38 SUMMARY

	TEMPERATURE	HUMIDITY
MEAN	70.5	41.0
MIN	68.5	8.0
MAX	73.1	58.5
SD	0.77	2.81
N SAMPLES	1733	1733
FIRST DAY	12/04/07	
LAST DAY	02/14/08	
N DAYS	73	

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT
HUMIDITY UNITS = % RELATIVE HUMIDITY
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES

REPORT 5
VERSION 1.10
4/1/2008 13:31

STUDY 189207 SUMMARY

MEAN	70.5	41.0
MIN	68.5	8.0
MAX	73.1	58.5
SD	0.77	2.81
N SAMPLES	1733	1733
FIRST DAY	12/04/07	
LAST DAY	02/14/08	
N DAYS	73	

NOTE: TEMPERATURE UNITS = DEGREES FAHRENHEIT
HUMIDITY UNITS = % RELATIVE HUMIDITY
NOTE: MEANS REPRESENT THE MEAN OF ALL VALUES

A 28-Day Oral (Gavage) Toxicity Study
Of H-28397 In Mice With A 28-Day Recovery
DuPont-24459
909
WIL-189207

APPENDIX G

Clinical Pathology Methods, Procedures And References

CLINICAL PATHOLOGY METHODS, PROCEDURES AND REFERENCES

Serum Chemistry - Hitachi 912

Albumin - Bromcresol Green (BCG) Method, Modification of the Doumas reaction. Default unit: g/dL. Hitachi 912 Application Code 413. Roche Reagent, catalog number 11970569.

A/G Ratio - Calculated from Albumin and Globulin Results. Default unit: %.

Alkaline Phosphatase - based on the International Federation of Clinical Chemistry (IFCC) Method (Tietz *et al.* J Clin Chem Clin Biochem 1983; 21:731-748). Default unit: U/L. Hitachi 912 Application Code 158. Roche Reagent, catalog number 2172933.

Alanine Aminotransferase - Modification of the International Federation of Clinical Chemistry (IFCC) recommended method. Default unit: U/L. Hitachi 912 Application Code 706. Roche Reagent, catalog number 450065.

Aspartate Aminotransferase - Kinetic method based on the International Federation of Clinical Chemistry (IFCC) recommendations. Default unit: U/L. Hitachi 912 Application Code 715. Roche Reagent, catalog number 450064.

Bilirubin (Total) - Diazo method developed by Wahlefeld *et al*. Scand J Clin Lab Invest 1972; 29: Supplement 126. Default unit: mg/dL. Hitachi 912 Application Code 716. Roche Reagent, catalog number 1039034.

Blood Urea Nitrogen (BUN) - A urease-triggered methodology based upon the method of Talke and Schubert Klin Wschr, 1965; 43:174. Default unit: mg/dL. Hitachi 912 Application Code 427. Roche Reagent, catalog number 1489321.

Calcium - Modified method using a gamma-amino-butyric acid (GABA) buffer. Default unit: mg/dL. Hitachi 912 Application Code 180. Roche Reagent, catalog number 1125621.

Chloride - An ion-selective electrode that measures the electrical potential of the ions present in solution. Default unit: mEq/L. Hitachi 912 Application. Roche Reagent, catalog numbers 820638, 836246 and 820639.

Cholesterol - Enzymatic reaction as described by Trinder. Ann Clin Biochem 1974; 12:266. Default unit: mg/dL. Hitachi 912 Application Code 722. Roche Reagent, catalog number 450061.

Creatinine - Modified Jaffe reaction based on the work of Poper *et al.* Biochem Z 1937; 291:354, and Seelig and Wuest. Aerztl Labor 1969; 15:34. Default unit: mg/dL. Hitachi 912 Application Code 727. Roche Reagent, catalog number 450019.

Globulin - Calculation obtained by subtracting Albumin from Total Protein. Default unit: g/dL.

Glucose - Glucose hexokinase method based on the work of Schmidt, Peterson and Young. Klin Wschr 1961; 39:1244. Methods of Enzymatic Analysis, 2nd Eng ed. New York, Academic Press, 1974; 1196. Anal Biochemistry 1958; 23:301. Default unit: mg/dL. Hitachi 912 Application Code 767. Roche Reagent, catalog number 450058.

Phosphorus - Method involves the formation of ammonium phosphomolybdate. Default unit: mg/dL. Hitachi 912 Application Code 714. Roche Reagent, catalog number 1040898.

Potassium - An ion-selective electrode that measures the electrical potential of the ions present in solution. Default unit: mEq/L. Hitachi 912 Application. Roche Reagent, catalog numbers 820638, 836246 and 820639.

Sodium - An ion-selective electrode that measures the electrical potential of the ions present in solution. Default unit: mEq/L. Hitachi 912 Application. Roche Reagent, catalog number 820638, 836246 and 820639.

Sorbitol Dehydrogenase (SDH) - An ultraviolet, kinetic method utilizing the following principal: Fructose + NADH $\xleftarrow{\text{SDH}}\longrightarrow$ Sorbitol + NAD. Default unit: U/L. Diagnostic Chemicals Limited, catalog number 740-25/740-10, Hitachi 912 Application.

Total Protein - Endpoint biuret method that utilizes a sample blank. Default unit: g/dL. Hitachi 912 Application Code 756. Roche Reagent, catalog number 1040901.

Triglycerides - Method that utilizes lipase from a microorganism to promote rapid and complete hydrolysis of triglycerides to glycerol. Default unit: mg/dL. Hitachi 912 Application Code 781. Roche Reagent, catalog number 1488899.

Hematology - Manual Methods

White Cell Differential - Manual method of counting 100 white cells stained with Wright Giemsa and entered on-line into the data files.

Reticulocyte Count - Manual method of counting the reticulocytes present in 1000 red blood cells stained with New Methylene Blue and entered on-line into the data files.

Red Blood Cell Morphology - Manual method of evaluating red blood cells on a Wright Giemsa-stained slide and entered on-line into the data files.

Platelet Estimate- Manual method of evaluating platelets on a Wright Giemsa-stained slide. Platelet estimation is evaluated and entered on-line into the data files as decreased, adequate or increased. Platelet clumps present on the slide will be reported as part of the RBC morphology.

Hematology-Bayer Advia® 120

WBC Count - The whole blood sample is mixed with ADVIA® 120 BASO reagent that contains acid and surfactant. The red cells are hemolyzed, and the white blood cells are then analyzed using two angle laser light scatter signals. Default unit: x 10^3 cells/μL

RBC / Platelet Count - Both red blood cells and platelets are analyzed by a single optical cytometer after appropriate dilution of the blood sample with ADVIA® 120 RBC/PLT reagent. The red blood cells are isovolumetrically sphered and lightly fixed with glutaraldehyde to preserve the spherical shape. Red cells and platelets are counted from the signals from a common detector with 2 different gain settings. Default unit RBC: x 10^6cells/μL. Default unit PLT: x 10^3 cells/μL

Hgb - Hemoglobin: The hemoglobin method is a modification of the manual cyanmethemoglobin method developed by the International Committee for standardization in Hematology (ICSH). Default unit: g/dL.

Hematocrit - The percentage of blood volume that is occupied by red blood cells. Also referred to as the packed red cell volume. On the ADVIA® 120 Hematology System this parameter is derived from the measured red cell volume (MCV) and the red cell count (RBC). Default unit: %.

MCH - Mean Corpuscular Hemoglobin: the average weight of hemoglobin in the red blood cells, calculated from the RBC and Hgb measurements. Default unit: pg.

MCHC - Mean Corpuscular Hemoglobin Concentration: the average concentration of hemoglobin in the red blood cells. This parameter is computed from the measured hemoglobin and the computed hematocrit. Default unit: g/dL.

MCV - Mean Corpuscular Volume: the average volume of the red blood cells. Default unit: Fl

White blood cell differential - The ADVIA® 120 Hematology System White Blood Cell Differential (WBC DIFF) methods, consists of both the Peroxidase method and the Basophil/Lobularity method. The ADVIA® 120 Hematology System performs a six-part differential that consists of basophils, eosinophils, large unstained cells, lymphocytes, monocytes, and neutrophils. The white blood cell differential count is reported in percent and the actual number of each type of cell per microliter of blood.

Reticulocyte - This method uses a nucleic acid dye (oxazine 750) to stain cellular RNA. The ADVIA® 120 autoRETIC reagent isovolumetrically spheres the erythroid cells and stains cellular RNA. Low-angle laser light scatter, high-angle laser light scatter, and absorption characteristics of all cells are counted and measured. The absorption data are used to classify each cell as a reticulocyte or mature red blood cell based on its RNA content. The reticulocyte is reported in percent and actual number x 10^9cells/Liter = thous/µl.

References:

ADVIA® 120 Hematology System Operator's Guide: Copyright© 1997, 1998 Bayer Corporation.

APPENDIX H

Pathology Report (WIL Research Laboratories, LLC)

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY OF H-28397 IN MICE WITH A 28-DAY RECOVERY

PATHOLOGY REPORT

Pathology Department

WIL Research Laboratories, LLC

TABLE OF CONTENTS

Page

Page

1. INTRODUCTION

The objective of this study was to evaluate the potential toxicity, and recovery there from, of H-28397 (a peroxisome proliferator) when administered to mice by oral gavage for 28 consecutive days.

2. STUDY DESIGN

Male and female Crl:CD-1(ICR) mice were administered vehicle or H-28397 via oral gavage once daily for 28 consecutive days as indicated in the following table. The dosage volume was 10 mL/kg for all groups.

Group Number	Test Substance	Dosage Level (mg/kg/day)	Number of Animals [a] Males	Females
1	Vehicle [b]	0	20	20
2	H-28397	0.1	10	10
3	H-28397	3	10	10
4	H-28397	30	20	20

[a] = 10 animals/sex/group were euthanized following a minimum of 28 days of dose administration; the remaining $\leq$ 10 animals/sex in Groups 1 and 4 were euthanized following a 28-day nondosing (recovery) period.

[b] = The vehicle was deionized water.

3. METHODS

3.1. CLINICAL PATHOLOGY

Hematology and serum chemistry parameters were evaluated on all animals just prior to the scheduled necropsies (i.e., 10 animals/sex/group at the primary necropsy and ≤ 10 animals/sex/group for Groups 1 and 4 at the recovery necropsy). Blood was collected for hematology and serum chemistry evaluation via the retro-orbital sinus of animals anesthetized by inhalation of isoflurane. The anticoagulant was potassium EDTA for hematology parameters. Anticoagulants were not used for serum chemistry samples.

The following parameters were evaluated.

3.1.1. HEMATOLOGY

Total leukocyte count (White Cells)
Erythrocyte count (Red Cells)
Hemoglobin
Hematocrit
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Platelet)
Reticulocyte count
  Percent (Reticulocyte)
  Absolute (Retic Absolute)

Differential leukocyte count -
  Percent and absolute
    -Neutrophil
    -Lymphocyte
    -Monocyte
    -Eosinophil
    -Basophil
    -Large unstained cell
Blood smears[a]

( ) - Designates tabular abbreviation
[a] - Blood smears were evaluated if scientifically warranted. Parameters evaluated from these smears included a differential leukocyte count, platelet estimates and RBC morphology.

3.1.2. SERUM CHEMISTRY

Total cholesterol (Cholesterol)
Triglycerides (Triglyceride)
Sorbitol dehydrogenase (Sorbitol'Genase) [a]
Alanine aminotransferase (Alanine Transfer)
Alkaline phosphatase (AlkalinePhos'tse)
Total bilirubin (Total Bili)
Total protein
Albumin
Globulin [by calculation]
Albumin/globulin ratio (A/G Ratio) [by calculation]
Aspartate aminotransferase (AspartatTransfer)
Urea nitrogen
Creatinine
Glucose
Calcium
Sodium
Potassium
Chloride
Phosphorus

( ) - Designates tabular abbreviation
[a] - Presented on special chemistry tables
Note: serum chemistry parameters are listed in order of priority for analysis

3.2. ANATOMIC PATHOLOGY

3.2.1. MACROSCOPIC EXAMINATION

Complete postmortem examinations were performed on all animals found dead or at the scheduled necropsies. At the scheduled necropsies, animals were euthanized by carbon dioxide inhalation and exsanguinated. At the time of necropsy, the following tissues and organs were collected and placed in 10% neutral-buffered formalin fixative unless otherwise noted:

Adrenals (2)
Aorta
Bone with marrow
  Femur
  Sternum
Bone marrow smear [a]
Brain
  Cerebrum (2 levels)
  Cerebellum with pons/medulla
Cervix
Epididymides (2) [b]
Exorbital lacrimal glands (2)
Eyes with optic nerves (2) [c]
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Peyer's patches
  Cecum
  Colon
  Rectum
Heart
Kidneys (2)
Larynx
Liver (sections of 2 lobes) [d]
Lungs (including bronchi, fixed by inflation with fixative)
Lymph nodes
  Mandibular
  Mesenteric
Nasal cavity [e]
Ovaries (2) with oviducts [f]
Pancreas
Peripheral nerve (sciatic)
Pharynx
Pituitary
Prostate
Salivary glands [mandibular (2)]
Seminal vesicles (2)
Skeletal muscle (rectus femoris)
Skin with mammary gland [g]
Spinal cord (cervical, thoracic, lumbar)
Spleen
Testes (2) [b]
Thymus
Thyroids [with parathyroids (2)] [f]
Tongue
Trachea
Urinary bladder
Uterus and vagina
Gross lesions and masses(when possible)

[a] - Bone marrow smears were obtained at the scheduled necropsies but not placed in formalin.
[b] - Fixed in Bouin's solution
[c] - Fixed in Davidson's solution
[d] - Representative cross-sections were collected from the left and median lobes at the time of necropsy and fixed in 10% neutral-buffered formalin.
[e] - Levels I and III were examined (Young, 1981).
[f] - Oviducts and parathyroids were examined microscopically when in the plane of section and in all cases where a gross lesion was present.
[g] - For females; a corresponding section of skin was collected from the same anatomic area for males.

3.2.2. ORGAN WEIGHTS

The following organs were weighed from all animals at the scheduled necropsies:

Adrenals	Ovaries (with oviducts)
Brain	Spleen
Epididymides	Testes
Heart	Thymus
Kidneys	Uterus
Liver	

Paired organs were weighed together. Organ-to-final-body-weight and organ-to-brain-weight ratios were calculated.

3.2.3. MICROSCOPIC EXAMINATION

Microscopic examination of routinely prepared hematoxylin-eosin stained paraffin sections was performed on all tissues collected at necropsy from all animals found dead and in the control and high dose groups euthanized at the scheduled primary necropsy. Gross lesions and target tissues, including the liver, adrenal glands, ovaries, oviducts, uterus, cervix and vagina, were examined from animals in the low- and mid-dose groups euthanized at the primary necropsy and animals in the control and high-dose groups euthanized at the recovery necropsy. Stained histologic sections were examined by light microscopy and observations were entered in the WIL Toxicology Data Management System (WTDMS™) by the pathologist. All gross necropsy observations were addressed and a cause-of-death/debility determination was made for all animals that died prior to scheduled study termination. Histologic sections were of adequate size and quality for detailed evaluation. The number of tissues examined from each dosage group may not necessarily equal the number of animals included in the group due to sectioning difficulties. The number of missing tissues was negligible and did not interfere with detection of test substance-related histologic alterations in the study. Histopathologic lesions were classified using standard published terminology to the extent possible. The

WTDMS™ histopathology tables contain all of the recorded data and serve as the basis for this narrative report.

3.3. ABBREVIATIONS

The following abbreviations may apply to this report:

Interval - point in the study at which event occurred (specimen collection, necropsy, etc.)
FD - found dead
PN - study day 28 primary necropsy (end of dosing)
RN - study day 56 recovery necropsy

3.4. DATA INTERPRETATION

In the discussion of clinical pathology parameters, values derived from the control group animals at all time points evaluated were considered as concurrent control values for purposes of constructing a 'normal' range for the present study. In addition, historical control values for this laboratory were consulted to refine data interpretation. Unless otherwise stated in this report, the 'normal' historical control range was represented by values within the WIL Historical control reference range (essentially a 95% confidence interval).

In the discussion of organ weight changes, the indication of higher or lower mean organ weights refers to a statistically significant ($p<0.05$ or $p<0.01$ using Dunnett's test) difference between test substance-treated versus control group animals in the present study. In addition, historical control values for this laboratory were consulted to refine data interpretation.

4. RESULTS

4.1. SURVIVAL

There were 2 unscheduled deaths during the course of this study. One control group female (no. 91015) was found dead on study day 25. Gross necropsy observations for this animal were limited to autolysis of multiple tissues including the intestinal tract, pancreas, and mesenteric lymph nodes. Histologic findings included lymphoid depletion in the spleen and thymus and depletion of the sternal and femoral bone marrow. One 30 mg/kg/day group female (no. 90987) was found dead on study day 9. There were no gross necropsy observations for this animal. Histologic findings included hepatocellular hypertrophy and lymphoid depletion in the thymus. Based on the gross necropsy and histologic findings, the cause of death in these 2 animals was undetermined.

4.2. CLINICAL PATHOLOGY

4.2.1. HEMATOLOGY AND COAGULATION

4.2.1.1. CHANGES ASSOCIATED WITH TEST SUBSTANCE ADMINISTRATION

There were no alterations in hematologic parameters that were considered adverse or directly to test substance administration.

Statistically significant decreases in red cell mass parameters (red blood cells, hemoglobin and/or hematocrit) were present in the 3 and 30 mg/kg/day group males. The changes in red cell mass parameters were minimal (mean values for all red cell mass parameters in these groups were decreased by approximately 8% or less compared to the respective control values). Based on the minimal nature of these changes, they were not considered to be adverse. Changes in the erythron showed recovery by study week 8. Effects on the erythron are summarized below in Text Table 1. In females, no effect on red cell mass was evident.

A slight increase in monocyte counts was present in the 30 mg/kg/day group males at study week 4. The relationship to treatment for this change is uncertain, however, it was

not considered adverse or toxicologically important because of the slight degree of change. There were no statistically significant changes in monocyte counts following the 4-week recovery period.

Text Table 1. Selected Hematology Findings - Males

Analysis	Group (mg/kg/day):	0	0.1	3	30
Red Cells (mil/µL)					
Week 4 Mean		8.80	8.44	8.28	8.13*
% Difference			-4.1	-5.9	-7.6
Week 8 Mean		9.21	NA	NA	9.38
% Difference					1.8
Hemoglobin (g/dL)					
Week 4 Mean		14.1	13.8	13.4*	13.1**
% Difference			-2.1	-5.0	-7.1
Week 8 Mean		15.0	NA	NA	14.7
% Difference					-2.0
Hematocrit (%)					
Week 4 Mean		40.1	38.8	38.1*	37.5**
% Difference			-3.2	-5.0	-6.5
Week 8 Mean		43.4	NA	NA	41.9
% Difference					-3.5

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

4.2.1.2. CHANGES UNRELATED TO TEST SUBSTANCE ADMINISTRATION

There were no other test substance-related effects on hematology (including coagulation) parameters. However, some statistically significant ($p<0.05$ or $p<0.01$ using Dunnett's test) differences were observed when the control and test substance-treated groups were compared. These findings included higher white blood cell counts, platelet counts and absolute lymphocyte counts and lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the 30 mg/kg/day group males on study week 8. These increases were not considered test substance-related because they occurred only at the recovery interval. Higher large unstained cell (LUC) counts in the 30 mg/kg/day males at study week 4 were not considered toxicologically important. When microscopic correlations were done, the vast majority of LUC were lymphoid cells, so higher LUC counts were most likely a result of higher lymphocyte counts, also present in the 30 mg/kg/day group males at study week 4, although not statistically significant.

Statistically significant findings that involved percentage reticulocyte or leukocyte differential counts were not itemized above and were not considered toxicologically important because absolute cell counts are utilized for interpretative purposes.

4.2.2. SERUM CHEMISTRY

4.2.2.1. CHANGES ASSOCIATED WITH TEST SUBSTANCE ADMINISTRATION

Alterations in serum chemistry parameters that were considered to be related to test substance administration are summarized below in Text Tables 2 and 3. Parameters that are toxicologically significant and test substance-related are **bolded** for further emphasis. Test substance-related serum chemistry findings that were toxicologically important included higher liver enzyme levels (alanine aminotransferase [ALT], alkaline phosphatase [ALP] and sorbitol dehydrogenase [SDH]) in the 3 and 30 mg/kg/day group males and 30 mg/kg/day group females at study week 4. Aspartate aminotransferase (AST) levels were also higher and toxicologically important in the 3 and 30 mg/kg/day group males at study week 4. These liver enzyme level changes were consistent with

hepatocellular injury (Solter, 2005) and single cell necrosis was noted microscopically in some animals in these groups. Liver enzyme changes were reversible in both males and females, as levels for all liver enzymes were similar to controls following 4 weeks of recovery. The only statistically significant change in liver enzymes in recovery group animals was a slight increase in sorbital dehydrogenase (SDH) in the 30 mg/kg/day recovery group males. However, the group mean for this group was similar to that of the control group mean at the end of dosing, and there were no correlative changes in other liver enzymes and no microscopic changes in the livers of animals in the 30 mg/kg/day recovery group males. Therefore, this slight increase in SDH was likely spurious and nonadverse. One animal in the 30 mg/kg/day recovery group males (no. 90941) had minimal increases in SDH and alanine aminotransferase, but the remaining liver enzymes were within the study control ranges. In addition, there were no microscopic changes in the liver of this animal. The basis of the slight increases in SDH and ALT in this animal was not determined.

Higher albumin, lower globulin, and associated changes of increased total protein and increased albumin/globulin ratio were present in the 30 mg/kg/day group males. Decreased globulin and increased albumin/globulin ratio were also present in the 3 mg/kg/day group males. A similar pattern of change in serum proteins was present in females administered 3 or 30 mg/kg/day. Decreases in acute phase proteins, which make up part of the serum globulin fraction are known to occur following treatment with other peroxisome proliferators (Gervois et al., 2004). Therefore, the changes in globulin were considered to be treatment-related. However, individual globulin values in all affected groups were within or, in a few animals, slightly outside the WIL historical control range. Therefore, the changes in globulin were not considered to be adverse. Similarly, increased albumin, a negative acute phase protein, has also been reported following treatment with peroxisome proliferators (Gervois et al., 2004). Therefore, the increases in albumin were also considered to be test substance-related. Except in the 30 mg/kg/day

group males, individual values for albumin in most animals were within the WIL historical control range. In the 30 mg/kg/day group males, albumin values for most animals were above the WIL historical control range. However, there are no reported adverse biological effects associated with increased albumin. Therefore, changes in albumin were not considered to be adverse. The changes in albumin and globulin were reversible, as there were no statistically significant changes in these parameters in males or females by study week 8.

Blood urea nitrogen (BUN) was slightly increased in the 30 mg/kg/day group males at the end of exposure. The increase in urea nitrogen in 30 mg/kg/day group males was minimal, was not associated with correlative changes in serum creatinine or with test substance-related microscopic findings in the kidney, and thus was considered to be nonadverse. Blood urea nitrogen was similar to control values following the 4-week recovery period. Modest increases in BUN have been reported to occur following treatment with a peroxisome proliferating compounds (Sheikh et al., 2006).

A statistically significant decrease in cholesterol was present in the 3 mg/kg/day group males. This decrease was not dose-related, as mean cholesterol in the 30 mg/kg/day group males was not statistically different from controls and was higher than that of the 3 mg/kg/day group males. However, several individual cholesterol values in treated group males were below the study control range, and thus, a test substance-related effect of decreased cholesterol can not be ruled out. However, individual cholesterol values in treated groups were within the WIL historical control range (with the exception of 1 male in the 3 mg/kg/day group) and thus changes in cholesterol were not considered to be adverse.

Text Table 2. Selected Serum Findings - Males					
Analysis	**Group (mg/kg/day):**	**0**	**0.1**	**3**	**30**
Albumin (g/dL)					
Week 4 Mean		3.2	3.2	3.3	**4.2****
% Difference			0.0	3.1	31.3
Week 8 Mean		3.2	NA	NA	3.2
% Difference					0.0
Total Protein (g/dL)					
Week 4 Mean		5.3	5.2	5.0	**6.0****
% Difference			-1.9	-5.7	13.2
Week 8 Mean		5.5	NA	NA	5.5
% Difference					0.0
Globulin (g/dL)					
Week 4 Mean		2.1	2.1	**1.7****	**1.8****
% Difference			0.0	-19.0	-14.3
Week 8 Mean		2.3	NA	NA	2.4
% Difference					4.3
A/G ratio (units)					
Week 4 Mean		1.54	1.56	**1.92****	**2.32****
% Difference			1.3	24.7	50.6
Week 8 Mean		1.43	NA	NA	1.34
% Difference					-6.3
Urea Nitrogen (mg/dL)					
Week 4 Mean		20.1	19.3	22.3	**24.5***
% Difference			-4.0	10.9	21.9
Week 8 Mean		24.4	NA	NA	21.6
% Difference					-11.5
Alkaline Phosphatase (U/L)					
Week 4 Mean		88	73	**144**	**1163****
% Difference			-17.0	63.6	1221.6
Week 8 Mean		55	NA	NA	63
% Difference					14.5

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Text Table 2. Selected Serum Findings - Males (continued)				
Analysis **Group (mg/kg/day):**	**0**	**0.1**	**3**	**30**
Alanine Aminotransferase (U/L)				
Week 4 Mean	52	38	**82**	**704****
% Difference		-26.9	57.7	1253.8
Week 8 Mean	72	NA	NA	86
% Difference				19.4
Aspartate Aminotransferase (U/L)				
Week 4 Mean	72	72	**90**	**416****
% Difference		0.0	25.0	477.8
Week 8 Mean	107	NA	NA	88
% Difference				-17.8
Sorbitol Dehydrogenase (U/L)				
Week 4 Mean	24	22	**46**	**456****
% Difference		-8.3	91.7	1800.0
Week 8 Mean	18	NA	NA	25*
% Difference				38.9
Cholesterol (mg/dL)				
Week 4 Mean	147	127	110**	131
% Difference		-13.6	-25.2	-10.9
Week 8 Mean	155	NA	NA	146
% Difference				-5.8

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Text Table 3. Selected Serum Findings - Females					
Analysis	**Group (mg/kg/day):**	**0**	**0.1**	**3**	**30**
Albumin (g/dL)					
Week 4 Mean		3.6	3.4	3.5	3.8*
% Difference			-5.6	-2.8	5.6
Week 8 Mean		3.2	NA	NA	3.1
% Difference					-3.1
Total Protein (g/dL)					
Week 4 Mean		5.4	5.2	5.2	5.3
% Difference			-3.7	-3.7	-1.9
Week 8 Mean		5.6	NA	NA	5.0
% Difference					-10.7
Globulin (g/dL)					
Week 4 Mean		1.9	1.8	**1.6****	**1.5****
% Difference			-5.3	-15.8	-21.1
Week 8 Mean		2.4	NA	NA	1.9
% Difference					-20.8
A/G Ratio (units)					
Week 4 Mean		1.93	1.98	**2.20****	**2.46****
% Difference			2.6	14.0	27.5
Week 8 Mean		1.64	NA	NA	1.75
% Difference					6.7
Alkaline Phosphatase (U/L)					
Week 4 Mean		90	97	96	**216****
% Difference			7.8	6.7	140.0
Week 8 Mean		64	NA	NA	73
% Difference					14.1
Alanine Aminotransferase (U/L)					
Week 4 Mean		52	38	63	**83**
% Difference			-26.9	21.2	59.6
Week 8 Mean		40	NA	NA	44
% Difference					10.0

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Text Table 3. Selected Serum Findings – Females (continued)				
Analysis **Group (mg/kg/day)**:	**0**	**0.1**	**3**	**30**
Sorbitol Dehydrogenase (U/L)				
Week 4 Mean	14	16	16	**40****
% Difference		14.3	14.3	185.7
Week 8 Mean	12	NA	NA	14
% Difference				16.7

NA = Not applicable
* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

4.2.2.2. CHANGES UNRELATED TO TEST SUBSTANCE ADMINISTRATION

There were no other test substance-related effects on serum chemistry parameters. However, some statistically significant ($p<0.05$ or $p<0.01$ using Dunnett's test) differences were observed when the control and test substance-treated groups were compared. These findings included slightly lower chloride levels in the 30 mg/kg/day group males at study week 4; this slight decrease was not considered toxicologically important because of the small magnitude of change (-0.9%).

4.3. GROSS OBSERVATIONS

The gross necropsy observations presented in the following table were considered to be related to administration of the test substance:

Text Table 4. Toxicologically Relevant Gross Necropsy Observations

Organ	Observation	Dosage Level(s) (mg/kg/day)	Sex	Interval(s)
Liver	Enlarged (A)	30	Male	PN
Histologic correlate	(A) hypertrophy, hepatocellular			

Descriptions of test substance-related histologic alterations are discussed in Section 4.5. (Histologic Changes).

4.4. ORGAN WEIGHTS

There were no test substance-related alterations in final body weight. Organ weight changes presented in the following table were considered to be associated with administration of the test substance:

Text Table 5. Toxicologically Relevant Final Body Weight And Organ Weight Changes

Parameter	Direction and magnitude of change	Dosage level (mg/kg/day)	Sex	Interval
Adrenal Glands				
Absolute	↑ 28.3%, ↑ 63.0%**			
Relative to body weight	↑ 30.8%, ↑ 69.2%**	3, 30	Male	PN
Relative to brain weight	↑ 27.9%, ↑ 64.3%**			
Liver				
Absolute	↑ 75.1%**, ↑ 166.3%**			
Relative to body weight	↑ 77.6%**, ↑ 163.1%**	3, 30	Male	PN
Relative to brain weight	↑ 76.2%**, ↑ 173.2%**			
Liver				
Absolute	↑ 34.1%**, ↑ 108.1%**			
Relative to body weight	↑ 32.1%**, ↑ 102.7**	3, 30	Female	PN
Relative to brain weight	↑ 28.5%**, ↑ 104.9%**			
Liver				
Absolute	↑ 21.0%*			
Relative to body weight	↑ 21.5%**	30	Male	RN
Relative to brain weight	↑ 21.8%*			

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Text Table 5. Toxicologically Relevant Final Body Weight And Organ Weight Changes (continued)

Parameter	Direction and magnitude of change	Dosage level (mg/kg/day)	Sex	Interval
Liver				
Absolute	↑ 20.0%			
Relative to body weight	↑ 14.3%*	30	Female	RN
Relative to brain weight	↑ 18.7%			
Uterus				
Absolute	↓ 39.1%*			
Relative to body weight	↓ 40.5%**	30	Female	PN
Relative to brain weight	↓ 40.3%*			

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Liver weights were increased in the 3 and 30 mg/kg/day group males and females at the end of the exposure period. These changes correlated with hepatocellular hypertrophy microscopically and with increases in beta-oxidation. Liver weight changes were mostly, but not completely, reversible in the 30 mg/kg/day group males and females. At this dose, liver weight relative to body weight in the 30 mg/kg/day group males was increased by 163.1% above controls at the end of exposure and was reduced to 21.5% of control after the 4-week recovery period. Similarly, in the 30 mg/kg/day group females, liver weight relative to body weight was increased by 102.7% above controls at the end of exposure and was reduced to 14.3% of control after the 4-week recovery period.

Adrenal gland weights (absolute and relative to body and brain weights) were increased in the 3 and 30 mg/kg/day group males at the end of the exposure period. In the 30 mg/kg/day male group, these adrenal weight changes correlated with minimal adrenal cortical hypertrophy microscopically. Adrenal weight changes were reversible following the 4-week recovery period.

Decreased uterus weights (absolute and relative to body and brain weights) were present in the 30 mg/kg/day group females at the end of the exposure period. There were no histopathological changes in the uterus that were correlative to the uterine weight changes. However, more animals in the 30 mg/kg/day group were in diestrus (based on vaginal histology) compared to controls (see discussion under Histologic Changes), and uterine weights are typically less during diestrus compared to estrus or proestrus. In the recovery groups, uterine weights were similar among treated and control groups.

There were no other test substance-related effects on organ weights. However, some statistically significant ($p<0.05$ or $p<0.01$ using Dunnett's test) differences were observed when the control and test substance-treated groups were compared. At the primary necropsy, there were higher mean absolute kidneys weights in the 0.1 (↑ 11.0%) and 30 (↑ 20.6%) mg/kg/day group females, higher mean kidneys weight relative to final body weight in the 0.1 (↑ 8.3%) and 30 (↑ 17.4%) mg/kg/day group females, and higher mean kidneys weight relative to brain weight in the 30 (↑ 19.1%) mg/kg/day group females. At the primary necropsy, there were higher mean absolute spleen weight (↑ 27.3%) and mean spleen weight relative to final body weight (↑ 23.6%) in the 0.1 mg/kg/day group females. The absolute weights and weights relative to body or brain weight were discordant or the dose association was incoherent; thus these organ weight changes were considered to be spurious. At the recovery necropsy, there were higher mean absolute adrenal gland weight (↑ 21.0%) and higher mean adrenal gland weight relative to brain weight (↑ 20.9%) in the 30 mg/kg/day group females. The individual animal adrenal gland weights for the 30 mg/kg/day recovery group females were similar to the individual animal adrenal gland weights for the primary control group females, and there were no gross or histologic correlates to the adrenal glands weight changes; thus these organ weight changes were considered to be spurious.

4.5. HISTOLOGIC CHANGES

4.5.1. CHANGES ASSOCIATED WITH TEST SUBSTANCE ADMINISTRATION

The following histologic changes observed at the study day 28 primary necropsy were considered to be related to test substance administration:

Text Table 6. Incidence Of Selected Histopathologic Findings, Study Day 28 Primary Necropsy

	Males				Females			
Dosage (mg/kg/day):	**0**	**0.1**	**3**	**30**	**0**	**0.1**	**3**	**30**
Adrenal Cortex [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Hypertrophy	0	0	0	8	0	0	0	0
Minimal	-	-	-	8	-	-	-	-
Liver [a]	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
Hypertrophy, hepatocellular	0	0	10	10	0	0	10	10
Minimal	-	-	0	0	-	-	8	0
Mild	-	-	8	0	-	-	2	0
Moderate	-	-	2	10	-	-	0	10
Increased mitoses	0	0	0	9	0	0	0	5
Minimal	-	-	-	6	-	-	-	2
Mild	-	-	-	1	-	-	-	3
Moderate	-	-	-	2	-	-	-	0
Necrosis, single cell	0	0	4	10	0	0	0	4
Minimal	-	-	4	10	-	-	-	4
Vagina [a]	**NA**	**NA**	**NA**	**NA**	**10**	**10**	**10**	**10**
Estrous cycle: Diestrus	NA	NA	NA	NA	3	6	5	10
Estrous cycle: Proestrus	NA	NA	NA	NA	2	2	3	0
Estrous cycle: Estrus	NA	NA	NA	NA	5	2	2	0

[a] = Number of tissues examined from each group.

Minimal adrenal cortical hypertrophy was observed in the 30 mg/kg/day group males at the primary necropsy. This change correlated with increased adrenal gland weights in this group. Adrenal cortical hypertrophy was not observed in the 30 mg/kg/day group males at the recovery necropsy.

Hepatocellular hypertrophy was observed in the 3 and 30 mg/kg/day group males and females at the primary necropsy. This change was consistent with increased liver weights

noted in these groups. The hepatocellular hypertrophy was characterized by expansion of the hepatocellular cytoplasm by numerous fine eosinophilic granules lending a generalized eosinophilic tinctorial change to the affected livers. The distribution of the hepatocellular hypertrophy was centrilobular when of minimal or mild severity and diffuse when of moderate severity. In addition, there was multifocal single cell hepatocellular necrosis in the 3 and 30 mg/kg/day group males and 30 mg/kg/day group females at the primary necropsy and increased mitoses distributed multifocally throughout the liver section in the 30 mg/kg/day group males and females at the primary necropsy with a higher incidence of these changes in the males compared to the females. Hepatocellular hypertrophy, single cell hepatocellular necrosis and increased mitoses in the liver were not observed in the 30 mg/kg/day group males and females at the recovery necropsy.

There was an increased number of animals in the diestrus stage of the estrous cycle in the 30 mg/kg/day group females compared to control group females at the primary necropsy. While this finding could be indicative of potential effects on the estrous cycle, ovarian morphology including number and maturational stages of corpora lutea were similar between treated and control groups, suggesting normal estrous cycling. Therefore, the significance of the differences in estrous stage distribution within the 30 mg/kg/day group females and control group females is uncertain. The number of animals in the diestrus stage of the estrous cycle was equal in the control and 30 mg/kg/day group females at the recovery necropsy.

4.5.2. CHANGES UNRELATED TO TEST SUBSTANCE ADMINISTRATION

At the primary necropsy, subacute inflammation in the region of the aorta, esophagus, pharynx, and/or thymus was observed in 1 control group male (no. 90953), one 30 mg/kg/day group male (no. 90902), 1 control group female (no. 90995), and three 30 mg/kg/day group females (nos. 90983, 91026, and 91040); necrosis and neutrophilic

infiltration in the region of the thymus was observed in 1 control group female (no. 91018). These histologic findings were consistent with gavage injuries.

At the recovery necropsy, pyogranulomatous inflammation and foreign material were observed in the adipose tissue and bronchial lymph node of 1 control group female (no. 90984) and in the region of the esophagus, mammary gland and skin in one 30 mg/kg/day group female (no. 91007). Reactive plasmacytosis was identified in the bronchial, mandibular, and/or mediastinal lymph nodes in these 2 animals. These histologic findings could be consistent with gavage injuries or other traumatic injury introducing foreign material into the tissues with subsequent pyogranulomatous inflammation.

There were no other test substance-related histologic changes. Remaining histologic changes were considered to be incidental findings or related to some aspect of experimental manipulation other than administration of the test substance. There was no test substance-related alteration in the prevalence, severity or histologic character of those incidental tissue alterations.

5. DISCUSSION

Relationships were suspected between gross necropsy, organ weight, clinical pathology and histopathology observations, as presented in the following table. These proposed relationships were based on subjective interpretation rather than a statistical analysis of correlation.

Text Table 7. Correlations Of Selected Observations

Necropsy	Organ Weight	Clinical Pathology	Histopathology
-	↑ adrenal gland weight (males)	-	Adrenal cortex- hypertrophy (males)
Liver- enlarged	↑ liver weight	↑ Alkaline phosphatase and SDH	Liver- hepatocellular hypertrophy
-	-	↑ ALT	Liver- single cell necrosis
-	↓ uterus weight	-	Vagina- estrous cycle disturbance

= no correlate

ALT = Alanine aminotransferase

SDH = Sorbitol dehydrogenase

Higher adrenal gland weights were noted in the 3 and 30 mg/kg/day group males and correlated to adrenal cortical hypertrophy in the 30 mg/kg/day group males at the primary necropsy.

Enlarged liver was noted in the 30 mg/kg/day group males at the primary necropsy and correlated to higher liver weights in the 3 and 30 mg/kg/day group males and females at the primary necropsy, elevated liver-related clinical pathology parameters, and the histologic finding of hepatocellular hypertrophy in the 3 and 30 mg/kg/day group males and females at the primary necropsy.

Lower uterus weight was noted in the 30 mg/kg/day group females at the primary necropsy. While there was no histologic evidence of uterine changes to directly correspond to the lower uterus weights, there was histologic evidence of estrous cycle disturbances based on examination of vaginal sections. The 30 mg/kg/day group females were all in the diestrus stage of the estrous cycle at the primary necropsy.

6. CONCLUSIONS

H-28397 was administered to 7 to 8 week old male and female Crl:CD-1 (ICR) mice via once daily oral (gavage) administration at dosage levels of 0 (control), 0.1, 3, and 30 mg/kg/day for 28 consecutive days followed by a 28-day nondosing recovery period in the remaining control and 30 mg/kg/day group animals.

There were 2 unscheduled deaths during the course of this study. One control group female (no. 91015) was found dead on study day 25 and one 30 mg/kg/day group female, (no. 90987) was found dead on study day 9. Based on the gross necropsy and histologic findings, the cause of death in these 2 animals was undetermined.

Most test substance-related effects were consistent with a peroxisome proliferator (PPARα agonist) and included increased liver weights, hepatocellular hypertrophy, and changes in serum lipids and proteins. Other changes included minimal decreases in red cell mass parameters and increased adrenal weights and adrenal cortical hypertrophy. These effects were reversible following a 4-week recovery period. Adverse effects were noted in the liver of males at 3 mg/kg/day and above and females at 30 mg/kg/day. These consisted of single cell necrosis of hepatocytes and correlative increases in liver enzymes. These effects were also reversible following the 4-week recovery period.

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A 28-Day Oral (Gavage) Toxicity Study
Of H-28397 In Mice With A 28-Day Recovery DuPont-24459

8. REPORT SUBMISSION

Report Submitted By:

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27Aug2008
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27 Aug 2008
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Reviewing Pathologist

27 Aug 2008
Date

APPENDIX I

Liver Metabolic Enzyme Analyses (Sponsor-Provided Data)

TRADE SECRET

Biochemical Measurements Report for
A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery

TEST GUIDELINES: OECD Guideline for the Testing of Chemicals
Section 4 (Part 407) (1995)

AUTHOR: Suzanne I. Snajdr, B.S.

BIOCHEMICAL MEASUREMENTS REPORT COMPLETED ON: May 20, 2008

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
DuPont Haskell Global Centers for
Health & Environmental Sciences
P.O. Box 50
Newark, Delaware 19714
U.S.A.

LABORATORY PROJECT ID: DuPont-24459

WORK REQUEST NUMBER: 17568

SERVICE CODE NUMBER: 1317

SPONSOR: E.I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The work performed at DuPont Haskell was conducted in compliance with EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with current OECD Good Laboratory Practices

Principal Investigator: _____ 20 May 2008
Suzanne I. Snajdr, B.S. Date
Associate Scientist

QUALITY ASSURANCE STATEMENT

Work Request Number: 17568
Service Code Number: 1317

Phase Audited	*Audit Dates*	*Date Reported to Study Director*	*Date Reported to Management*
Conduct:	March 11, 2008	March 18, 2008	March 18, 2008
Report/Records:	May 09,13, 2008	May 13, 2008	May 14, 2008

Reported by: Donna M. Johnston 20 May 2008
Donna M. Johnston
Quality Assurance Auditor
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Biochemical Measurements Evaluation by: Suzanne I. Snajdr, B.S.
Associate Scientist

20 May 2008
Date

SUMMARY

H-28397 was evaluated for its ability to alter hepatic peroxisomal β-oxidation activity, a measure of peroxisome proliferation, and total hepatic microsomal cytochrome P-450 enzyme content in male and female mice following approximately 28 days of oral (gavage) administration of 0, 0.1, 3 or 30 mg/kg/day or after approximately 28 days of recovery.

In male mice, β-oxidation activity was statistically significantly increased at the 28-day time point at dosages of 0.1, 3 and 30 mg/kg/day H-28397 and total cytochrome P-450 content was statistically significantly decreased at dosages of 3 and 30 mg/kg/day H-28397. The increases in hepatic β-oxidation at 3 and 30 mg/kg/day H-28397 were accompanied with increases in relative liver weights. In female mice dosed with 3 and 30 mg/kg/day H-28397, β-oxidation activity and relative liver weights were statistically significantly increased at the 28-day time point while total cytochrome P-450 content remained unaltered. β-oxidation activity had returned to control levels after approximately 28 days of recovery while the relative liver weights remained statistically elevated at 30 mg/kg/day H-28397 in both male and female mice. Total cytochrome P-450 content had remained statistically decreased after approximately 28 days of recovery in male mice.

Under the conditions of this study, H-28397 was an inducer of hepatic peroxisomal β-oxidation activity, a measure of peroxisome proliferation, in male mice after administration of 0.1, 3 and 30 mg/kg/day and in female mice after administration of 3 and 30 mg/kg/day of 28 days oral gavage. H-28397 is a peroxisome proliferator at these dosage levels. Total hepatic microsomal cytochrome P-450 enzyme content was decreased at a dosage of 3 and 30 mg/kg/day of 28 days oral gavage in male mice but not in females. β-oxidation activity in both male and female mice had returned to control levels after approximately 28 days of recovery while total cytochrome P-450 content remained below control levels in the males.

MATERIALS AND METHODS

A. Biochemical Analysis

Following approximately 28 days (test day 29) of oral (gavage) administration of 0, 0.1, 3 or 30 mg/kg/day or after approximately 28 days of recovery (test day 59), 10 mice from each sex/group designated for biochemical evaluation were weighed and then euthanized by CO_2 anesthesia and exsanguination at WIL Research Laboratory, LLC (Ashland, Ohio). The livers were removed, weighed, a portion of each was flash frozen in liquid nitrogen, stored frozen (approximately -60 ^{o}C to -80 ^{o}C) and shipped to DuPont Haskell on dry ice. The liver portions were then stored frozen (approximately -60 ^{o}C to -80 ^{o}C) until homogenized (approximately 1 gram tissue/8 mL buffer) in homogenization buffer (50 mM Tris-HCl, 50 mM Trizma-base, 0.25 M sucrose, and 5.4 mM EDTA, pH 7.4). Hepatic peroxisomes and microsomes were prepared using differential centrifugation. The resulting peroxisomal and microsomal pellets were resuspended in the homogenization buffer, aliquoted, and stored between -60 and -80°C until analyzed. The peroxisomal suspensions were diluted to a protein concentration of approximately 0.25 mg/mL, and β-oxidation activity was determined using [^{14}C]palmitoyl CoA as the substrate.(1) The microsomal suspensions were diluted to a protein concentration of approximately 1.0 mg/mL, and the total cytochrome P-450 content were measured by spectral analysis according to the method of Omura and Sato.(2) The spectra were recorded at room temperature with a spectrophotometer. The protein content of the peroxisomes and microsomes were determined before and after analysis by the Biorad method.(3) Final calculations for the rate of β-oxidation and total cytochrome P-450 content were made using the post-assay protein concentrations.

RESULTS AND DISCUSSION

Mechanistic Evaluation

(Tables 1-6, Appendix A)

H-28397 was evaluated for its ability to alter hepatic peroxisomal β-oxidation activity, a measure of peroxisome proliferation, and total hepatic microsomal cytochrome P-450 enzyme content in male and female mice following approximately 28 days of oral (gavage) administration of 0, 0.1, 3 or 30 mg/kg/day H-28397 or after approximately 28 days of recovery.

In male mice at the 28-day time point, β-oxidation activity was statistically significantly increased at dosages of 0.1, 3 and 30 mg/kg/day H-28397 (157, 844 and 748% of control, respectively). Percent liver weight relative to body weight was statistically significantly increased at dosages of 3 and 30 mg/kg/day H-28397 (178 and 263% of control, respectively). Total cytochrome P-450 content was statistically significantly decreased at a dosage of 3 and 30 mg/kg/day H-28397 (74 and 56% of control, respectively). β-oxidation activity returned to the control level at the 28-day recovery time point while the relative liver weight remained statistically increased at 30 mg/kg/day H-28397 (122% of control). Total cytochrome P-450 content remained decreased at 30 mg/kg/day H-28397 (88% of control) after approximately 28 days of recovery.

In female mice at the 28-day time point, β-oxidation activity was statistically significantly increased at dosages of 3 and 30 mg/kg/day H-28397 (595 and 923% of control, respectively). Percent liver weight relative to body weight was statistically significantly increased at dosages of 3 and 30 mg/kg/day H-28397 (132 and 203% of control, respectively). No alteration in total cytochrome P-450 content was observed. β-oxidation activity returned to the control level at the 28-day recovery time point while the relative liver weight remained statistically increased at 30 mg/kg/day H-28397 (114% of control).

Under the conditions of this study, H-28397 was an inducer of hepatic peroxisomal β-oxidation activity, a measure of peroxisome proliferation, in male mice after administration of 0.1, 3 and 30 mg/kg/day and in female mice after administration of 3 and 30 mg/kg/day of 28 days oral gavage. H-28397 is a peroxisome proliferator at these dosage levels. Total hepatic microsomal cytochrome P-450 enzyme content was decreased at a dosage of 3 and 30 mg/kg/day of 28 days oral gavage in male mice but not in females. β-oxidation activity in both male and female mice had returned to control levels after approximately 28 days of recovery while total cytochrome P-450 content remained below control levels in the males

RECORDS AND SAMPLE STORAGE

Specimens (if applicable), raw data, the protocol, amendments (if any), and the final report will be retained at DuPont Haskell, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware.

Laboratory-specific raw data such as personnel files, instrument, equipment, refrigerator and/or freezer raw data will be retained at the facility where the work was done.

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TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

n number of samples
NA not applicable
SD standard deviation

Table 1
Summary of Hepatic Peroxisomal Beta-Oxidation Activity in Male Mice

		Hepatic Peroxisomal Beta-Oxidation Activity (nmol/min/mg protein)			
	Dosage	28-Day		28-Day Recovery	
Group[a]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	8.1	2.6	11.0	4.2
2	0.1	12.7[b]	3.2	--[c]	--
3	3	68.4[b]	11.5	--	--
4	30	60.6[b]	7.4	12.0	2.5

a n=10.
b Statistically significant difference from control at $p < 0.05$ by Dunnett's test.
c Group not analyzed.

Table 2
Summary of Hepatic Peroxisomal Beta-Oxidation Activity in Female Mice

		Hepatic Peroxisomal Beta-Oxidation Activity (nmol/min/mg protein)			
	Dosage	28-Day[a]		28-Day Recovery[b]	
Group	(mg/kg/day)	Mean	SD	Mean	SD
1	0	8.3	3.6	10.2	1.8
2	0.1	9.6	2.4	--[c]	--
3	3	49.4[d]	12.0	--	--
4	30	76.6[d]	10.4	10.6	3.8

a n=10.
b n=9.
c Group not analyzed.
d Statistically significant difference from control at $p < 0.05$ by Dunn's test.

Table 3
Summary of Percent Liver Weight Relative to Body Weight in Male Mice

		Relative Liver Weight (%)[a]			
	Dosage	28-Day		28-Day Recovery	
Group[b]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	4.816	0.4493	4.966	0.3361
2	0.1	5.282	0.3908	--[c]	--
3	3	8.555[d]	1.0672	--	--
4	30	12.669[d]	1.8178	6.035[e]	0.9227

a Data supplied by WIL Research Laboratories, LLC project number WIL-189207.
b n=10.
c Group not analyzed.
d Statistically significant difference from control at p < 0.05 by Dunn's test.
e Statistically significant difference from control at p < 0.05 by Dunnett's test.

Table 4
Summary of Percent Liver Weight Relative to Body Weight in Female Mice

		Relative Liver Weight (%)[a]			
	Dosage	28-Day[b]		28-Day Recovery[c]	
Group	(mg/kg/day)	Mean	SD	Mean	SD
1	0	4.785	0.4267	4.787	0.6245
2	0.1	5.156	0.3002	--[d]	--
3	3	6.321[e]	0.6333	--	--
4	30	9.699[e]	1.2157	5.473[f]	0.7008

a Data supplied by WIL Research Laboratories, LLC project number WIL-189207.
b n=10.
c n=9.
d Group not analyzed.
e Statistically significant difference from control at p < 0.05 by Dunn's test.
f Statistically significant difference from control at p < 0.05 by Dunnett's test.

Table 5
Summary of Hepatic Microsomal Total Cytochrome P450 Content in Male Mice

		Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)			
	Dosage	28-Day		28-Day Recovery	
Group[a]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	0.808	0.124	0.849	0.090
2	0.1	0.775	0.131	--[b]	--
3	3	0.600[c]	0.056	--	--
4	30	0.454[c]	0.035	0.751[c]	0.099

a n=10.
b Group not analyzed.
c Statistically significant difference from control at $p < 0.05$ by Dunnett's test.

Table 6
Summary of Hepatic Microsomal Total Cytochrome P450 Content in Female Mice

		Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)			
	Dosage	28-Day		28-Day Recovery	
Group[a]	(mg/kg/day)	Mean	SD	Mean	SD
1	0	0.632	0.152	--[b]	--
2	0.1	0.643	0.136	--	--
3	3	0.592	0.096	--	--
4	30	0.555	0.105	--	--

a n=10.
b Group not analyzed.
There were no statistically significant differences from control at $p < 0.05$ by Dunnett's or Dunn's test.

Appendix A
Individual Animal Data

INDIVIDUAL ANIMAL DATA

EXPLANATORY NOTES

ABBREVIATIONS:

NA - not analyzed
NS - no sample

FOOTNOTES:
a Data supplied by WIL Research Laboratories, LLC project number WIL-189207.

28-Day Male Mice

Animal Number	Group	Dose (mg/kg)	Hepatic Peroxisomal Beta-Oxidation Rate (nmol/mg-min)	Relative Liver Weight (%)[a]	Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)
90915	1	0	8.7	5.014	0.696
90925	1	0	4.0	4.855	0.684
90929	1	0	10.5	5.303	0.727
90939	1	0	6.2	5.132	0.729
90940	1	0	6.9	4.368	0.698
90943	1	0	8.2	4.030	0.978
90953	1	0	7.9	5.291	0.930
90959	1	0	10.0	5.216	0.987
90961	1	0	13.1	4.446	0.892
90965	1	0	5.8	4.503	0.762
90908	2	0.1	9.7	5.125	0.575
90914	2	0.1	10.1	4.899	0.764
90924	2	0.1	12.2	5.800	0.739
90934	2	0.1	11.0	4.762	0.604
90938	2	0.1	7.6	4.810	0.659
90946	2	0.1	13.2	5.788	0.870
90951	2	0.1	13.7	5.636	0.899
90954	2	0.1	15.6	5.519	0.968
90956	2	0.1	16.2	5.231	0.842
90963	2	0.1	17.8	5.246	0.831
90907	3	3	60.9	7.013	0.587
90913	3	3	58.3	7.212	0.649
90919	3	3	67.5	8.047	0.577
90927	3	3	84.7	8.441	0.503
90948	3	3	52.1	8.121	0.584
90949	3	3	86.0	9.349	0.616
90960	3	3	73.1	8.842	0.686
90966	3	3	72.0	10.645	0.570
90967	3	3	56.9	8.596	0.670
90969	3	3	72.6	9.281	0.555
90902	4	30	66.5	9.534	0.465
90904	4	30	53.2	11.337	0.510
90906	4	30	65.2	16.038	0.417
90920	4	30	69.6	11.196	0.478
90926	4	30	63.4	13.600	0.397
90931	4	30	66.8	13.239	0.451
90950	4	30	47.1	13.707	0.430
90952	4	30	51.9	13.724	0.444
90955	4	30	61.0	12.750	0.452
90962	4	30	61.6	11.560	0.500

28-Day Female Mice

Animal Number	Group	Dose (mg/kg)	Hepatic Peroxisomal Beta-Oxidation Rate (nmol/mg-min)	Relative Liver Weight (%)[a]	Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)
90986	1	0	7.4	4.871	0.493
90992	1	0	6.0	4.688	0.509
90995	1	0	5.9	5.249	0.433
90997	1	0	4.9	4.462	0.574
91000	1	0	7.6	5.196	0.499
91002	1	0	7.0	4.860	0.657
91016	1	0	10.8	4.125	0.772
91018	1	0	17.2	4.215	0.880
91019	1	0	6.8	4.794	0.714
91037	1	0	9.1	5.391	0.789
90977	2	0.1	6.5	4.796	0.506
90981	2	0.1	8.7	5.571	0.574
90996	2	0.1	9.2	5.354	0.493
90998	2	0.1	9.2	5.102	0.504
91011	2	0.1	6.0	5.384	0.581
91020	2	0.1	13.2	5.062	0.757
91031	2	0.1	9.0	5.294	0.790
91033	2	0.1	10.0	4.844	0.824
91035	2	0.1	11.8	4.704	0.592
91038	2	0.1	12.5	5.449	0.810
90973	3	3	39.1	5.896	0.523
90988	3	3	39.5	6.145	0.475
90990	3	3	50.7	6.129	0.465
90993	3	3	58.2	6.092	0.554
91006	3	3	66.3	6.080	0.570
91013	3	3	55.7	5.923	0.667
91017	3	3	24.7	5.753	0.751
91023	3	3	56.6	7.171	0.662
91032	3	3	48.2	7.751	0.565
91034	3	3	55.3	6.269	0.692
90976	4	30	77.9	9.800	0.491
90978	4	30	73.8	10.617	0.447
90983	4	30	69.1	9.470	0.489
90991	4	30	83.4	12.237	0.426
91004	4	30	70.7	9.774	0.515
91012	4	30	78.0	9.865	0.697
91026	4	30	88.8	8.840	0.657
91027	4	30	89.4	9.986	0.625
91039	4	30	80.7	8.757	0.502
91040	4	30	54.3	7.646	0.705

28-Day Recovery Male Mice

Animal Number	Group	Dose (mg/kg)	Hepatic Peroxisomal Beta-Oxidation Rate (nmol/mg-min)	Relative Liver Weight (%)[a]	Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)
90903	1	0	5.7	5.068	0.735
90905	1	0	11.8	5.277	0.982
90911	1	0	4.6	5.018	0.861
90912	1	0	15.1	4.646	0.936
90917	1	0	14.4	5.167	0.910
90922	1	0	18.0	5.140	0.831
90932	1	0	11.2	4.748	0.733
90942	1	0	9.1	4.207	0.910
90945	1	0	11.1	5.136	0.859
90958	1	0	8.8	5.251	0.731
90910	4	30	8.6	6.430	0.822
90923	4	30	13.4	5.536	0.840
90930	4	30	13.2	6.448	0.720
90935	4	30	12.4	7.657	0.851
90937	4	30	11.6	5.255	0.808
90941	4	30	15.1	7.237	0.829
90944	4	30	11.5	5.947	0.698
90947	4	30	13.8	4.692	0.631
90964	4	30	6.7	5.860	0.559
90968	4	30	13.2	5.286	0.748

28-Day Recovery Female Mice

Animal Number	Group	Dose (mg/kg)	Hepatic Peroxisomal Beta-Oxidation Rate (nmol/mg-min)	Relative Liver Weight (%)[a]	Hepatic Microsomal Total Cytochrome P450 Content (nmol/mg protein)
90975	1	0	9.4	5.468	NA
90984	1	0	8.3	5.287	NA
90985	1	0	8.1	5.507	NA
90999	1	0	9.6	5.087	NA
91015	1	0	NS	NS	NA
91021	1	0	12.6	4.216	NA
91024	1	0	8.8	4.971	NA
91025	1	0	11.2	3.748	NA
91028	1	0	12.9	4.228	NA
91030	1	0	10.8	4.573	NA
90972	4	30	7.7	5.158	NA
90987	4	30	NS	NS	NA
91001	4	30	16.4	5.057	NA
91003	4	30	13.8	5.541	NA
91005	4	30	10.8	5.275	NA
91007	4	30	6.7	7.291	NA
91008	4	30	5.8	5.325	NA
91010	4	30	12.9	5.333	NA
91014	4	30	13.4	4.996	NA
91029	4	30	7.5	5.277	NA

APPENDIX J

Study Protocol

Study Number: WIL-189207

PROTOCOL AMENDMENT VII

Sponsor: E.I. du Pont de Nemours and Company

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in ~~Rats~~ * Mice① with a 28-Day Recovery

B. Protocol Modifications:

1) **12** **WORK PRODUCT:**

The first paragraph of this section of the protocol is revised to read as follows:

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including slides, specimens, raw paper data, pertinent electronic storage media, and leftover test substance will be returned to the Sponsor at the address on page 2 of this protocol at the time of issuance of the final report. Unless otherwise indicated, all remaining formulation and clinical pathology samples will not be sent to Archives and will be discarded at the time of the issuance of the final report or earlier.

* This should be mice cbc 8.18.08

① Clarification MCH 8/20/08

189207 ①
WIL-~~187207~~
Protocol Amendment VII
Page 2 of 2

C. Reasons for Protocol Modification:

1) Clarification of final disposition of study work product by request of the Sponsor.

Sponsor approval received via e-mail on 8/13/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter 8/14/08
Carol Carpenter Date
Sponsor Representative

WIL Research Laboratories, LLC

8/13/08
Matthew C. Haas, BA, LAT Date
Study Director

8/12/08
Christopher P. Chengelis, PhD, DABT Date
Director, Toxicology

① Correction of study number. MCH 8/27/08

Study Number: WIL-189207

PROTOCOL AMENDMENT VI

Sponsor: E.I. du Pont de Nemours and Company

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery

B. Protocol Modifications:

1) **8.7.4 Microscopic Examination:**

The microscopic slides for this study will be shipped to the Sponsor at the address below for Sponsor's internal review, including taking of photomicrographs of the slides. This Sponsor review is only for Sponsor's internal use, is not to be considered a peer review and will not be conducted under GLP compliance. Results of this internal review and/or the photomicrographs of slides will not be included in the Final Report for this study.

Shipping address:
Carolyn Lloyd
Haskell Laboratory for Health & Environmental Sciences
1090 Newark Elkton Road
Bldg S320
Newark, DE 19714

WIL-189207
Protocol Amendment VI
Page 2 of 2

C. Reasons for Protocol Modification:

1) Slide shipment and Sponsor's internal review scheduled by request of the Sponsor.

Sponsor approval received via e-mail on 6/9/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 6/12/08
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

Matthew C. Haas, BA, LAT, Study Director — 6/10/08 Date

Christopher P. Chengelis, PhD, DABT, Director, Toxicology — 17 June 2008 Date

Study Number: WIL-189207

PROTOCOL AMENDMENT V

Sponsor: E.I. du Pont de Nemours and Company

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery

B. Protocol Modifications:

1) **1 OBJECTIVE:**

The United States Environmental Protection Agency (EPA) Good Laboratory Practice Regulations that this study will be conducted in compliance with should be 40 CFR Part 792, September 18, 1989.

2) **3 STUDY SCHEDULE:**

Proposed Experimental Termination Date: April 10, 2008

C. Reasons for Protocol Modification:

1) Clarification of EPA Regulation study conducted in compliance with.

WIL-189207
Protocol Amendment V
Page 2 of 2

2) The Proposed Experimental Termination Date was added based upon the last histopathological examination.

Sponsor approval received via e-mail on 5.20.08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter 6-9-08
Carol Carpenter Date
Sponsor Representative

WIL Research Laboratories, LLC

5.28.08
Matthew C. Haas, BA, LAT Date
Study Director

28 May 2008
Christopher P. Chengelis, PhD, DABT Date
Director, Toxicology

Study Number: WIL-189207

PROTOCOL AMENDMENT IV

Sponsor: E.I. du Pont de Nemours and Company

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery

B. Protocol Modifications:

1) 3 **STUDY SCHEDULE:**

The fifth item in this section will be revised to read as follows:

Proposed Audited Report Date: May 30, 2008

C. Reasons for Protocol Modification:

1) The Proposed Audited Draft Report Date was re-scheduled following scheduling of the target tissue histopath and for an earlier available date per request of the Sponsor.

Sponsor approval received via e-mail on 4/3/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 7 Apr 2008
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

4/3/08
Matthew C. Haas, BA, LAT, Study Director — Date

3 April 08
Christopher P. Chengelis, PhD, DABT, Director, Toxicology — Date

Study Number: WIL-189207

PROTOCOL AMENDMENT III

Sponsor: E.I. du Pont de Nemours and Company

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery

B. Protocol Modifications:

1) **8.7.4 Microscopic Examination:**

Microscopic examination will be extended (at additional cost) to the organs/tissues listed below in the low- and mid-dose groups euthanized at the primary necropsy and the control and high-dose groups euthanized at the recovery necropsy since the organs/tissues listed below were identified as potential target organs based on histopathological examination of tissues from the control and high dose groups or other parameters (organ weights, clinical pathology, etc.).:

-Liver
-Adrenal glands
-Ovaries with oviducts
-Cervix
-Uterus and vagina

C. Reasons for Protocol Modification:

1) Additional histopathological examination on potential target organs requested by Sponsor.

Sponsor approval received via e-mail on 3/12/08.
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 25-Mar-2008
Carol Carpenter
Sponsor Representative
Date

WIL Research Laboratories, LLC

Matthew C. Haas, BA, LAT — 3/12/08
Matthew C. Haas, BA, LAT
Study Director
Date

Christopher P. Chengelis, PhD, DABT — 12 Mar 2008
Christopher P. Chengelis, PhD, DABT
Director, Toxicology
Date

Study Number: WIL-189207

PROTOCOL AMENDMENT II

Sponsor: E.I. du Pont de Nemours and Company

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery

B. Protocol Modifications:

1) **2.2** **WIL Study Director:**

Effective February 15, 2008 this section will be revised to read as follows:

Matthew C. Haas, BA, LAT
Staff Toxicologist
Tel: (419) 289-8700
Fax: (419) 289-3650
Email: mhaas@wilresearch.com

2) **2.6** **Principal Investigator – Pathology:**

This section will be revised to read as follows:

Amera K. Remick, DVM, DACVP
Pathologist
Biotechnics, Inc.
Tel: (919) 245-3114
Fax: (919) 245-3115
Email: aremick@biotechnics-inc.com

WIL-189207
Protocol Amendment II
Page 2

C. Reasons for Protocol Modification:

1) Change in Study Director.

2) The information became available.

Sponsor approval received via email on 11 Feb 2008
Date

E.I. du Pont de Nemours and Company

Carol Carpenter 15-Feb-2008
Carol Carpenter Date
Sponsor Representative

WIL Research Laboratories, LLC

13 Feb 2008
Michael S. Koch, PhD Date
Study Director

13 Feb 2008
Christopher P. Chengelis, PhD, DABT Date
Director, Toxicology

Study Number: WIL-189207

PROTOCOL AMENDMENT I

Sponsor: E.I. du Pont de Nemours and Company

A. Title of Study:

A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery

B. Protocol Modifications:

1) **3 STUDY SCHEDULE:**

The fifth item in this section will be revised to read as follows:

Proposed Audited Report Date: June 10, 2008

2) **7.4.1 Organization of Test Groups::**

In accordance with the Study Director Notification dated December 20 and 21, 2007 footnote "d" will be added to the tables in this section as follows:

Group Number	Treatment	Dosage Level[a] (mg/kg/day)	Dose Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle[b]	0	0	10	20[c]	20[c]
2	H-28397	0.1	0.01	10	10[c]	10[c]
3	H-28397	3	0.3	10[d]	10[c]	10[c]
4	H-28397	30	3	10	20[c]	20[c]

a - Dosage levels will be adjusted for purity using a correction factor of 1.14.
b - Deionized (DI) water
c - 10 animals/sex/group will be submitted for euthanasia at the primary necropsy on Day 28. The remaining animals (≤ 10 animals/sex/group) in Groups 1 and 4 will be euthanized at the recovery necropsy on Day 56.
d - Dose volume increased from 10 to 12 mL/kg for Week 0.

3) **7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Article Formulations:**

In accordance with the Study Director Notifications dated December 12, 14, and 19, 2007 the fourth sentence of this section will be revised to read as follows:

For resuspension homogeneity and stability analysis, two aliquots, similar in size to the amount required for one day of dosing (20 mL of the 0.01 mg/mL formulation and 30 mL of the 3 mg/mL formulation), from each preparation

for 5 hours
V
will be stored one each at room temperature or refrigerated (approximately 2° to 8° C) for 12 days.

4) **7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Article Formulations:**

In accordance with the Study Director Notifications dated December 12, 14, and 19, 2007 the second paragraph of this section will be deleted.

5) **7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Article Formulations:**

In accordance with the Study Director Notifications dated December 20 and 21, 2007 the third paragraph of this section will be revised to read as follows:

Four 1-mL samples will be collected from the middle stratum of each dose concentration (including controls) of each preparation of the dosing formulations for potential analysis of test article concentration. Samples from Weeks 0, 1 and 3 will be analyzed at WIL Research Laboratories, LLC according to a validated method.

6) **8.7.4 Microscopic Examination:**

The first paragraph of this section will be revised to read as follows:

Histologic preparation will be conducted at the WIL Research Laboratories subsidiary (Biotechnics) in Hillsborough, North Carolina, and documented in the raw data.

C. Reasons for Protocol Modification:

1) The Proposed Audited Draft Report Date was postponed to allow for incorporation of all ancillary reports into the main report prior to issuance.

2) The Group 3 Week 0 dosing formulation was below the acceptable range for concentration of a suspension formulation as defined by WIL Research Laboratories, LLC SOP. Accordingly, the dose volume was adjusted for the first week of the study to approximate administration of the protocol-specified dosage level for Group 3.

3) Due to difficulties experienced in formulating the pre-initiation batches for WIL-189205 stability data for formulations with a dose concentration of 0.01

V = Insert for clarification mch 5-28-08

mg/mL could not be established. Consequently, these analyses were added to WIL-189207.

4) To maintain consistence with the addition of stability analyses to the protocol.

5) To allow for collection of samples to verify the concentrations of all dosing formulations and to allow for concentration analysis of the Week 1 formulations.

6) To facilitate histological processing and histopathological evaluation the tissues will be prepared by Biotechnics.

Sponsor approval received via email on 24 Jan 2008
Date

E.I. du Pont de Nemours and Company

Carol Carpenter — 29-Jan-2008
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

Michael S. Koch, PhD, Study Director — 24 Jan 2008 Date

for Christopher P. Chengelis, PhD, DABT, Director, Toxicology — 24 Jan 2008 Date

PROTOCOL

A 28-DAY ORAL (GAVAGE) TOXICITY STUDY OF H-28397 IN MICE WITH A 28-DAY RECOVERY

OECD 407 Guidelines

Sponsor:

E.I. du Pont de Nemours and Company
Wilmington, DE 19898

Work Request, Service Code: **WR 17568, SC 1317**
DuPont study number: DuPont-24459

Performing Laboratory:

WIL Research Laboratories, LLC
1407 George Road
Ashland, OH 44805-8946

1 OBJECTIVE:

The objective of this study is to evaluate the potential toxicity, and recovery therefrom, of H-28397 when administered to mice by oral gavage for 28 consecutive days.

This study will be conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Regulations (40 CFR Part 160), October 16, 1989, the Organization for Economic Co-operation and Development Principles of Good Laboratory Practice [(C/97 186/Final], the Standard Operating Procedures of WIL Research Laboratories, LLC, and the protocol as approved by the Sponsor.

This study was designed in accordance with the OECD Guideline for the Testing of Chemicals 407 (Repeated Dose 28-Day Oral Toxicity Study in Rodents).

2 PERSONNEL INVOLVED IN THE STUDY:

2.1 Sponsor Representative:

Carol Carpenter
Senior Staff Toxicologist
DuPont Haskell Global Centers for
Health and Environmental Sciences
1090 Elkton Rd, PO Box 50
Newark, DE 19714
Tel: (302) 366-5201
Fax: (302) 366-5207
Email: Carol.Carpenter@usa.dupont.com

2.2 WIL Study Director:

Michael S. Koch, PhD
Staff Toxicologist
Tel: (419) 289-8700
Fax: (419) 289-3650
Email: mkoch@wilresearch.com

2.3 WIL Deputy Director:

Jason M. Roper, PhD
Staff Toxicologist
E-mail: jroper@wilresearch.com

2.4 WIL Departmental Responsibilities:

Christopher P. Chengelis, PhD, DABT
Director, Toxicology

Jozef J.W.M. Mertens, PhD, DABT
Associate Director, General Toxicology

George A. Parker, DVM, PhD, DACVP, DABT
Director, Pathology

Daniel W. Sved, PhD
Director, Metabolism and Analytical Chemistry

Philip L. Stetson, MD, PhD
Associate Director, Analytical Chemistry

Walter R. Miller, Jr., BS, DVM
Clinical Veterinarian, Head of Surgery
and Experimental Medicine

Susan C. Haley, BS
Manager, Clinical Pathology

Sally A. Keets, AS
Senior Operations Manager, Vivarium

Carol A. Kopp, BS, LAT
Manager, Gross Pathology and
Developmental Toxicology Laboratory

Teresa D. Morris, BS
Senior Operations Manager, Toxicology

Heather L. Johnson, BS, RQAP-GLP
Manager, Quality Assurance

Theresa M. Rafeld
Group Manager, Formulations Laboratory

Michael Safron, AS, HT (ASCP), CM
Manager, Histology

Robert A. Wally, BS, RAC
Manager, Reporting and Regulatory
Technical Services

Ronald E. Wilson, BS
Director, Informational Systems

2.5 Principal Investigator – Clinical Pathology Data Analysis:

Ellen L. Ziemer, DVM, PhD, DACVIM, DACVP
Senior Clinical Pathologist
Biotechnics LLC
310 Millstone Drive
Hillsborough, NC 27278
Tel: (919) 245-3114
Email: eziemer@wilresearch.com

2.6 Principal Investigator – Pathology:

To be added by protocol amendment.

3 STUDY SCHEDULE:

Proposed Animal Receipt Date:	December 4, 2007
Proposed Experimental Start Date:	December 18 & 19, 2007
Proposed Necropsy Dates	
Primary Necropsy:	January 15 & 16, 2008
Recovery Necropsy:	February 14, 2008
Proposed Experimental Termination Date:	To be added by Amendment
Proposed Audited Report Date:	May 9, 2008

4 TEST ARTICLE DATA:

4.1 Identification:

FRD-902 (or H-28397)

4.2 Haskell Test Substance Number:

H-28397

4.3 Lot Number:

E1131181-19-B

4.4 Purity:

The Certificate of Analysis (C of A) provided by the Sponsor indicates the test article is 88% pure. A copy of the C of A will be maintained in the study records. A factor of 1.14 will be used to correct for purity.

4.5 Stability:

The analysis was performed by the Sponsor, and documented on the Certificate of Analysis.

4.6 Physical Description:

To be documented by WIL Research Laboratories, LLC.

4.7 Storage Conditions:

Controlled room temperature and humidity (approximately 65° to 75°F and 20% to 70% relative humidity)

4.8 Reserve Samples:

Retention samples will be collected and stored in accordance with WIL Standard Operating Procedures.

4.9 Personnel Safety:

MSDS to be provided by Sponsor.

4.10 Test Article Disposition:

With the exception of the reserve sample for each batch of test article, which will be archived as described, all neat test article remaining at study completion will be returned to the Sponsor.

5 TEST SYSTEM:

5.1 Species:

Mouse

5.2 Strain:

Crl:CD-1(ICR)

5.3 Source:

Charles River Laboratories
(Facility to be documented in the raw data.)

5.4 Number of Animals:

Seventy males and 70 females will be ordered and 60 of each sex placed on study. Animals not utilized on study will be deemed as part of the stock colony or euthanized by CO_2 inhalation and discarded without necropsy.

5.5 Approximate Age and Weight:

Animals will be approximately 5 to 6 weeks of age when received, and approximately 7 to 8 weeks of age at initiation of dosing. Animals are expected to weigh approximately 25 to 40 g at the initiation of dosing. Females will be nulliparous and non-pregnant.

5.6 Identification System:

Each animal will be uniquely identified by a metal ear tag displaying the animal number. Individual cage cards will be affixed to each cage and will display the animal number, sex, group number and study number.

5.7 Justification for Selection:

This species and strain of animal is recognized as appropriate for toxicity studies. The CD-1 mouse will be utilized because it is a widely used strain and historical data are available. The number of animals selected is the minimum needed to yield statistically and scientifically meaningful data, and is consistent with regulatory guidelines.

6 SPECIFIC MAINTENANCE SCHEDULE:

6.1 Animal Housing:

Animals will be housed in an environmentally controlled room, three per cage, by sex, in clean, wire-mesh cages, for approximately three days following receipt. Animals may be housed two per cage, by sex, if the number of animals does not permit housing three per cage. Thereafter, all animals will be housed individually. The cages will be elevated above cage-board or other suitable

material. The cages will be subject to routine cleaning at a frequency consistent with maintaining good animal health.

The facilities at WIL Research Laboratories, LLC are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

6.2 Environmental Conditions:

Controls will be set to maintain an average daily temperature at 71 ± 5°F (22 ± 3°C) and an average daily relative humidity at approximately 30-70%. Temperature and relative humidity will be monitored continuously. Data for these two parameters will be scheduled for automatic collection on an hourly basis. Fluorescent lighting will provide illumination for a 12-hour light/dark photoperiod. Temporary adjustments to the light/dark cycles may be made to accommodate protocol-specified activities. The ventilation rate will be set at a minimum of 10 room air changes per hour, 100% fresh air.

6.3 Drinking Water:

Reverse osmosis-treated tap water will be available *ad libitum.* Filters servicing the automatic watering system will be changed regularly according to Standard Operating Procedures. Municipal water supplying the laboratory will be analyzed for contaminants to ascertain that none are present at concentrations that would be expected to affect the outcome of the study according to WIL Standard Operating Procedures.

6.4 Diet:

PMI Nutrition International, LLC Certified Rodent LabDiet® 5002 (Meal) will be offered *ad libitum.* Each lot utilized will be identified and recorded. Standard Operating Procedures provide specifications for acceptable levels of heavy metals and pesticides that are reasonably expected to be present in the diet without interfering with the purpose or conduct of the study. Each lot of feed has been analyzed to assure specifications are met. Copies of lot appropriate analyses will be included in the study records. Feeders will be changed and sanitized once per week.

7 EXPERIMENTAL DESIGN:

7.1 Animal Receipt and Acclimation:

Each animal will be inspected by qualified personnel upon receipt. Animals judged to be in good health will be placed immediately in acclimation for at least 10 days (including the pretest period). During the acclimation period, each

animal will be assigned a permanent animal number and observed twice daily for changes in general appearance and behavior. The animals will be allowed a pretest week (as part of the acclimation period) during which body weights and food consumption will be determined and general health will be monitored, but they will not receive test article. All animals will receive a detailed physical examination during the pretest period and at the time of selection for randomization.

7.2 Randomization:

Near the end of the pretest period, animals judged to be suitable for testing will be assigned to groups at random based on body weight stratification into a block design using a computer program. A printout containing the animal numbers and individual group assignments will be generated. Animals will then be arranged into the groups according to the printout. Body weights at randomization will be within ± 20% of the mean for each sex. If, after randomization, significant differences between groups exist, new randomizations will be generated until group mean body weights are not statistically significant between groups.

Following randomization but before dosing on study day 0, it may be necessary to replace individual animals. The replacement animal(s) will be arbitrarily selected from the remaining pre-test animals. The reason(s) for replacement will be appropriately documented in the study records.

7.3 Route and Rationale of Test Article Administration:

The route of administration will be oral, by gavage, since one of the study objectives is to determine the potential toxicity of the test article when administered by the oral route and further potential testing will be by the oral route.

7.4 Organization of Test Groups, Dosage Levels and Treatment Regimen:

7.4.1 Organization of Test Groups:

The dose levels were supplied by the Sponsor, and will be adjusted using a correction factor of 1.14 to correct for purity. The following table presents the study group arrangement:

Group Number	Treatment	Dosage Level[a] (mg/kg/day)	Dose Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Animals	
					Males	Females
1	Vehicle[b]	0	0	10	20[c]	20[c]
2	H-28397	0.1	0.01	10	10[c]	10[c]
3	H-28397	3	0.3	10	10[c]	10[c]
4	H-28397	30	3	10	20[c]	20[c]

a - Dosage levels will be adjusted for purity using a correction factor of 1.14.
b - Deionized (DI) water.
c - 10 animals/sex/group will be submitted for euthanasia at the primary necropsy on Day 28. The remaining animals (≤ 10 animals/sex/group) in Groups 1 and 4 will be euthanized at the recovery necropsy on Day 56.

7.4.2 Justification of Dosage Levels:

The dosage levels used on this study were selected by the Sponsor based upon existing toxicity data for this test article.

7.4.3 Treatment Regimen:

Vehicle or H-28397 formulations will be administered orally by gavage once daily for a minimum of 28 consecutive days (until the day prior to the scheduled necropsy). The first day of dosing will be designated as Day 0. Day 28 is the first day of the primary necropsy and the first day of recovery. Recovery animals will be held without dosing for a minimum of an additional 28 days. Day 56 is the day of the recovery necropsy. Group 1 animals will receive the vehicle and serve as controls.

7.4.4 Method of Administration:

The test article and vehicle formulations will be administered via stainless steel ball-tipped metal and PTFE plastic dosing cannulae and plastic syringes of appropriate size. Vehicle and test article formulations will be stirred continuously after preparation and throughout the dosing period.

7.4.5 Adjustment of Dosages:

Individual doses will be adjusted for the duration of the study, based on the most recent body weights. Adjusted doses will become effective on the day new body weights are recorded.

7.5 Preparation and Analysis of Test Article Dosing Formulations:

7.5.1 Storage and Method of Preparation of the Test Article:

Formulations of the test article in deionized water will be prepared approximately weekly, with daily aliquots stored refrigerated (approximately 2° to 8°C), and used within 10 days of preparation. The dose formulations will be placed on a stir plate for continuous stirring during sample collection and prior to dose administration. The dose formulations will be adjusted for purity using a correction factor of 1.14.

7.5.2 Homogeneity, Resuspension Homogeneity, Stability and Concentration Determination of Test Article Formulations:

Pre-initiation batches of H-28397 at concentrations of 0.01 mg/mL (batch size 150 mL) and 3 mg/mL (batch size 210 mL) will be prepared in a volume large enough to dose a group of animals for approximately one week. On the day of formulation, four 1-mL samples will be collected from each stratum (top, middle and bottom) of these formulations to determine the homogeneity of the batches. The samples from the middle stratum will also serve as confirmation of concentration samples. For resuspension homogeneity analysis, aliquots similar in size to the amount required for one day of dosing (20 mL of the 0.01 mg/mL formulation and 30 mL of the 3 mg/mL formulation) will be stored refrigerated for 10 days. After remixing at room temperature for a minimum of ten minutes using a magnetic stirrer, four 1-mL samples will be collected from the top and bottom strata of the formulations to assess resuspension homogeneity. Samples will be collected by WIL Research Laboratories Analytical Chemistry department personnel and analyzed at WIL Research Laboratories, LLC using a validated method (Koch, draft).

Stability analyses will not be performed. The results of prior stability analyses of H-28397, formulated in DI water, indicate the test article is stable for 5 hours at room temperature and for 10 days when refrigerated at approximately 2°-8°C (Koch, draft).

Four 1-mL samples will be collected from the middle stratum of each dose concentration (including controls) of the Week 0 and Week 3 dosing formulations for analysis of test article concentration. Samples will be analyzed at WIL Research Laboratories, LLC according to a validated method.

7.5.3 Analysis of Test Article Formulations:

Samples will be transferred to the Department of Analytical Chemistry at WIL Research Laboratories, LLC for analysis. Analyses of test article formulations will be performed using a method developed and validated by WIL Research Laboratories, LLC. Initially, two of each set of four replicate, 1-mL samples will be analyzed; the remaining two 1-mL samples will be stored frozen (approximately -10° to -30°C) at WIL and will function as back-up samples. Back-up samples will be analyzed if requested by the Sponsor or Study Director or may be discarded if the results are within specifications.

Results of the analyses will be provided to the Study Director, and included in the WIL Research Final Report.

8 PARAMETERS TO BE EVALUATED:

8.1 Viability Observations:

All animals will be observed for mortality/moribundity twice daily, once in the morning and once in the afternoon. Moribund animals will be euthanized and necropsied. Animals found dead will be necropsied as soon as possible to minimize the possibility of tissues being lost due to autolysis.

8.2 Animals to be Euthanized *in Extremis*:

All animals to be euthanized *in extremis* will receive a detailed physical examination and have a final body weight collected prior to release for necropsy. Additionally, an attempt will be made to collect blood samples for evaluation of hematology and serum chemistry parameters (see Clinical Pathology section), to aid in determining the cause of the animal's moribund condition. The animal will then be released for euthanasia by CO_2 inhalation and subsequent necropsy (see Anatomic Pathology section).

8.3 Clinical Observations:

8.3.1 Daily Observations:

A clinical examination will be performed daily for all animals at the time of dosing and approximately 1-2 hours after dosing, or once daily during the recovery period. Clinical examinations during the recovery period may be omitted on days of detailed physical examinations. Observations will include, but are not limited to, changes in the skin, fur, eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems function; somatomotor activity and behavior

patterns. The absence or presence of clinical findings at each scheduled observation period will be recorded for individual animals. Findings noted for individual animals outside of the specified observation periods will also be recorded.

8.3.2 Detailed Physical Examinations:

All animals will receive a detailed physical examination at least once during the pretreatment period, at randomization, at least weekly thereafter, and just prior to the scheduled necropsy. The animals will be removed from their home cages and placed in a standard arena for observations. Observations will be detailed and carefully recorded. Where appropriate, explicitly defined scoring systems will be used if, in the opinion of the Study Director, doing so increases the utility of the data. Signs noted shall include, but not be limited to, changes in the skin, fur, eyes, and mucous membranes, occurrence of secretions and excretions and autonomic activity (e.g., lacrimation, piloerection, pupil size, and unusual respiratory pattern). Changes in gait, posture and response to handling, as well as presence of clonic or tonic movements, stereotypic behavior (e.g., excessive grooming, repetitive circling) or bizarre behavior (e.g., self-mutilation, walking backwards) will be recorded. Signs such as skin lesions and hair loss will also be recorded at this time. The absence or presence of findings will be recorded for individual animals.

8.4 Individual Body Weights:

Individual body weights will be recorded at pretest initiation, at randomization, at least weekly during the treatment and recovery periods, and on the day of euthanasia.

8.5 Individual Food Consumption:

Individual food consumption will be recorded approximately weekly during the pretest period, prior to randomization, at least weekly during the treatment and recovery periods, and on the day prior to necropsy.

8.6 Clinical Pathology:

Blood samples for clinical pathology will be collected from all surviving animals on the day of their scheduled necropsy (i.e., 10 animals/sex/group at the primary necropsy and $\leq$ 10 animals/sex/group for Groups 1 and 4 at the recovery necropsy).

Blood samples for serum chemistry and hematology will be collected from the retro-orbital sinus of animals anesthetized by inhalation of isoflurane. The target volumes of the blood samples for serum chemistry and hematology analyses are 0.6 mL and 0.3 mL, respectively. In the event that insufficient blood can be collected to perform both analyses priority will be given to serum chemistry analysis.

The anticoagulant will be potassium EDTA for the hematology. Samples for serum chemistry will be collected without anticoagulants.

8.6.1 Serum Chemistry[a]:

Total cholesterol	Aspartate aminotransferase
Triglycerides	Urea nitrogen
Sorbitol dehydrogenase	Creatinine
Alanine aminotransferase	Glucose
Alkaline phosphatase	Calcium
Total bilirubin	Sodium
Total protein	Potassium
Albumin	Chloride
Globulin	Phosphorus
A/G ratio, calculated	

a - Serum chemistry parameters are listed in the order of priority.

8.6.2 Hematology:

Blood smears[a]	MCHC
Differential leukocyte count	MCV
Erythrocyte count	Platelet count
Hematocrit	Reticulocyte count
Hemoglobin	Total leukocyte count
MCH	

a - Blood smears will be made for all animals receiving a hematology evaluation as per WIL Research Laboratories, LLC SOPs T5-027, T5-085 and T5-139. The blood smear will only be evaluated if scientifically warranted (at additional cost). Parameters evaluated from these smears will include a differential leukocyte count, platelet estimates and RBC morphology.

8.7 <u>Anatomic Pathology:</u>

8.7.1 Macroscopic Examination:

A complete necropsy will be conducted on all animals dying spontaneously, euthanized *in extremis* or at the scheduled necropsies.

Animals euthanized *in extremis* or at study termination will be euthanized by carbon dioxide inhalation and exsanguinated. Necropsy will include examination of the external surface, all orifices and the cranial, thoracic, abdominal and pelvic cavities including viscera. At the time of necropsy the following tissues and organs will be collected and placed in 10% neutral-buffered formalin (except as noted):

Adrenals (2)
Aorta
Bone with marrow
  Femur
  Sternum
Bone marrow smear [a]
Brain
  Cerebrum Level 1
  Cerebrum Level 2
  Cerebellum with medulla/pons
Cervix
Epididymides (2)[c]
Exorbital lacrimal glands (2)
Eyes with optic nerve (2)[b]
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Peyer's patches
  Cecum
  Colon
  Rectum
Heart
Kidneys (2)
Larynx
Liver[g]
Lungs (including bronchi, fixed by inflation with fixative)
Lymph node
  Mandibular
  Mesenteric
Nasal cavity[d]
Ovaries (2) with oviducts[e]
Pancreas
Peripheral nerve (sciatic)
Pharynx
Pituitary
Prostate
Salivary glands [mandibular (2)]
Seminal vesicles (2)
Skeletal muscle (rectus femoris)
Skin with mammary gland[f]
Spinal cord
  Cervical
  Thoracic
  Lumbar
Spleen
Testes (2)[c]
Thymus
Thyroid [with parathyroids (2)[e]]
Tongue
Trachea
Urinary bladder
Uterus and vagina
All gross lesions and masses (when possible)

a - Not taken from animals found dead, not placed in formalin, only evaluated if scientifically warranted.
b - To be placed in Davidson's solution.
c - To be placed in Bouin's solution.
d - Levels I and III according to the method of Young (Young, 1981) will be examined.
e - Oviducts and parathyroids will be examined microscopically if in the plane of section and in all cases where a gross lesion of the organ is present.
f - For females; a corresponding section of skin will be taken from the same anatomic area for males.
g - Representative cross-sections will be collected from the left and median lobes at the time of necropsy and stored in 10% neutral-buffered formalin. The remaining liver tissue will be collected for liver metabolic enzyme analysis as indicated in Protocol Section 8.7.3.

8.7.2 Organ Weights:

The following organs from all animals euthanized at the scheduled necropsies will be weighed:

Adrenals	Ovaries (with oviducts)
Brain	Spleen
Epididymides	Testes
Heart	Thymus
Kidneys	Uterus
Liver	

Paired organs will be weighed together. Organ-to-body-weight and organ-to-brain-weight ratios will be calculated from animals euthanized at the scheduled necropsies.

8.7.3 Liver Metabolic Enzyme Analysis:

Following collection of the organ weights for all animals euthanized at the scheduled necropsies, an approximate 1-gram sample (if possible) of liver (the entire right and caudate lobes, and the tissue remaining from the left and median lobes after cross-sections are taken for histopathological examination) will be collected. The tissue will then be rinsed in chilled saline, placed in a plastic bag, flash frozen in liquid nitrogen and stored frozen (approximately -60° to -80°C) until shipped to DuPont Haskell for analysis. A liver tissue sample for metabolic enzyme analysis will not be collected from any animal euthanized *in extremis* or found dead. Frozen tissue samples will be shipped on dry ice, by overnight courier, to the address below:

Carol Carpenter
Senior Staff Toxicologist
DuPont Haskell Global Centers for
Health and Environmental Sciences
1090 Elkton Rd, PO Box 50
Newark, DE 19714
Tel: (302) 366-5201
Fax: (302) 366-5207
Email: Carol.Carpenter@usa.dupont.com

Liver samples will be analyzed for the following:

Total cytochrome P450 content
Beta oxidation activity

The results of these analyses will be provided to WIL Research Laboratories, LLC in the form of a GLP-compliant report, which will be appended to the final report.

8.7.4 Microscopic Examination:

Histologic preparation will be conducted at either the WIL Research Laboratory site in Ashland, Ohio or at the WIL Research Laboratories subsidiary (Biotechnics) in Hillsborough, North Carolina, and documented in the raw data. If the tissues are shipped to North Carolina, an appropriate protocol amendment will be issued prior to such shipment.

Microscopic examination of hematoxylin-eosin stained paraffin sections will be performed on the tissues/organs listed in the Macroscopic Examination Section from all animals found dead, euthanized *in extremis* and in the control and high dose groups euthanized at the scheduled primary necropsy. Gross lesions will be examined from animals in the low- and mid-dose groups euthanized at the primary necropsy and animals in the control and high-dose groups euthanized at the recovery necropsy. Microscopic examination may be extended to other organs/tissues from animals in the low- and mid-dose groups euthanized at the primary necropsy and the control and high-dose groups euthanized at the recovery necropsy (by protocol amendment, at additional cost) if a potential target organ is noted based on histopathological examination of tissues from the control and high dose groups or other parameters (organ weights, clinical pathology, etc.). Special stains may be used at the discretion of the pathologist to further characterize lesions and changes. Any special stains used will be documented in the individual animal data and interpretation of results will be included in the final report.

9 STATISTICAL METHODS:

All analyses except for liver metabolic analysis will be two-tailed for significance levels of 5% and 1%. Significance for liver metabolic analysis will be judged at $p < 0.05$. Statistical analysis will not be performed on groups with an N of two (2) or less. Separate analyses will be performed on the data collected for each sex. All means will be presented with standard deviations. All statistical tests will be performed using appropriate computing devices or programs. Body weights, body weight changes and food consumption as well as clinical pathology values (except gamma glutamyltransferase), and absolute and relative organ weights will be subjected to a one-way analysis of variance (Snedecor and Cochran, 1980). If a statistically significant difference ($p<0.05$) is present in this ANOVA, a comparison

of the control group to each treated group by Dunnett's test (Dunnett, 1964) will be performed.

Cytochrome P450 and beta oxidation data will be subjected to a preliminary test of homogeneity (Levene, 1960) and normality (Shapiro and Wilk, 1965). If the preliminary test is not significant, one-way analysis of variance (Snedecor and Cochran, 1980) followed by Dunnett's test (Dunnett, 1964; Tamhane, 1979) will be performed. If the preliminary test is significant, a Kruskal-Wallis test (Kruskal and Wallis, 1952) followed by Dunn's test (Dunn, 1964) will be performed.

10 QUALITY ASSURANCE:

The study will be audited by the WIL Quality Assurance Unit while in progress to assure compliance with Good Laboratory Practice regulations, adherence to the protocol and to WIL Research Laboratories, LLC Standard Operating Procedures. The raw data and draft report will be audited by the WIL Quality Assurance Unit to assure that the final report accurately describes the conduct and the findings of the study. Unless requested by the Sponsor, the WIL QAU will not audit the work performed by subcontractors or Sponsor. It is assumed that these organizations have independent QAUs and that they will be responsible for GLP compliance of their work.

This study is a GLP-compliant study and will be included on the WIL Research Laboratories, LLC master list of regulated studies.

Liver metabolic enzyme analysis will be audited by the DuPont Haskell Quality Assurance Unit.

11 RECORDS TO BE MAINTAINED:

All original raw data records, as defined by WIL SOPs and the applicable GLPs, will be returned to the Sponsor, as described in protocol section 12.

12 WORK PRODUCT:

Sponsor will have title to all documentation records, raw data, slides, specimens, or other work product generated during the performance of the study. All work product including raw paper data, pertinent electronic storage media, and leftover test substance will be returned to the Sponsor at the address on page 2 of this protocol. All specimens will be shipped directly to EPL Archives, Inc., Sterling, VA. Unless otherwise indicated, all remaining formulation and clinical pathology samples will not be sent to Archives and will be discarded at the time of the issuance of the final report.

Any work product, including documents, specimens, and samples, that are required by this protocol, its amendments, or other written instructions of the Sponsor, to be shipped by WIL Research Laboratories, LLC to another location will be appropriately packaged and labeled as defined by WIL's SOPs and delivered to a common carrier for shipment. WIL Research Laboratories, LLC will not be responsible for shipment following delivery to the common carrier.

13 REPORTS:

The final report will contain a summary, test substance data, methods and procedures, appropriate individual animal and summary data tables, a copy of the protocol and amendments (if any) and an interpretation and discussion of the study results. The report will contain all information necessary to conform to current OECD specifications. The report will be comprehensive and shall attempt to define the level(s) inducing toxic effects, as well as "no-effect" level(s) under the conditions of this investigation.

WIL Research Laboratories, LLC will provide one (1) copy of an Audited Draft Report, submitted in a timely manner upon completion of the study phase and prior to issuance of the final report. One (1) revision will be permitted as part of the cost of the study, from which Sponsor's reasonable revisions and suggestions will be incorporated into the Final Report as appropriate. Additional changes or revisions may be made, at extra cost. It is expected that the Sponsor will review the draft report and provide comments to WIL within a two (2) month time frame following submission. WIL will submit the Final Report within one (1) month following receipt of comments. If the Sponsor's comments and/or authorization to finalize the report have not been received at WIL within one year of submission of the draft report, WIL may elect to finalize the report following appropriate written notification to the Sponsor. The Final Report will be provided as a PDF (electronic) copy and in MS Word format (electronic copy).

14 PROTOCOL MODIFICATION:

Modification of the protocol may be accomplished during the course of this investigation. However, no changes will be made in the study design without the verbal or written permission of the Sponsor. In the event that the Sponsor verbally requests or approves changes in the protocol, such changes will be made by appropriate documentation in the form of protocol amendments. All alterations of the protocol and reasons for the modification(s) will be signed by the Study Director and the Sponsor Representative.

15 ANIMAL WELFARE ACT COMPLIANCE:

This study will comply with all applicable sections of the Final Rules of the Animal Welfare Act regulations (9 CFR). The Sponsor should make particular note of the following:

- The Sponsor signature on this protocol documents for the Study Director the Sponsor's assurance that the study described does not unnecessarily duplicate previous experiments
- Whenever possible, procedures used in this study have been designed to avoid or minimize discomfort, distress or pain to animals. All methods are described in this study protocol or in written laboratory Standard Operating Procedures.
- Animals that experience severe or chronic pain or distress that cannot be relieved will be painlessly euthanized, as deemed appropriate by the veterinary staff and Study Director. The Sponsor will be advised by the Study Director of all circumstances which could lead to this action, in as timely a manner as possible.
- Methods of euthanasia used during this study are in conformance with the above-referenced regulation.
- The Sponsor/Study Director has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals and has provided a written narrative description (AWA covered species) of the methods and sources used to determine that alternatives are not available.

16 REFERENCES:

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17 PROTOCOL APPROVAL:

Sponsor approval via email on 30 Nov 2007.

E.I. du Pont de Nemours and Company

Carol Carpenter — 4 Dec 2007
Carol Carpenter, Sponsor Representative — Date

WIL Research Laboratories, LLC

Michael S. Koch, PhD, Study Director — 3 Dec 2007 — Date

Christopher P. Chengelis, PhD, DABT, Director, Toxicology — 3 Dec 2007 — Date